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VACCINATION

PLATE I.

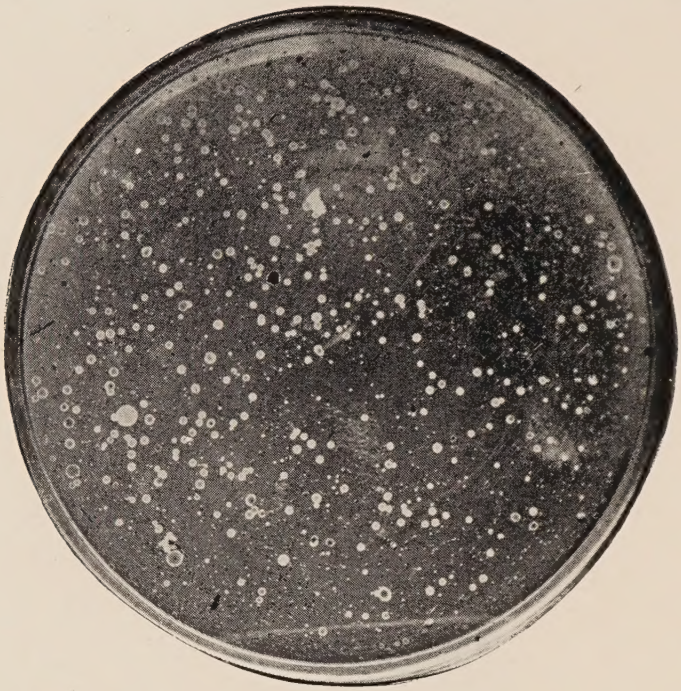

FIG. 1.—Photograph of agar plate prepared with vaccine material *immediately* after GLYCERINATION.

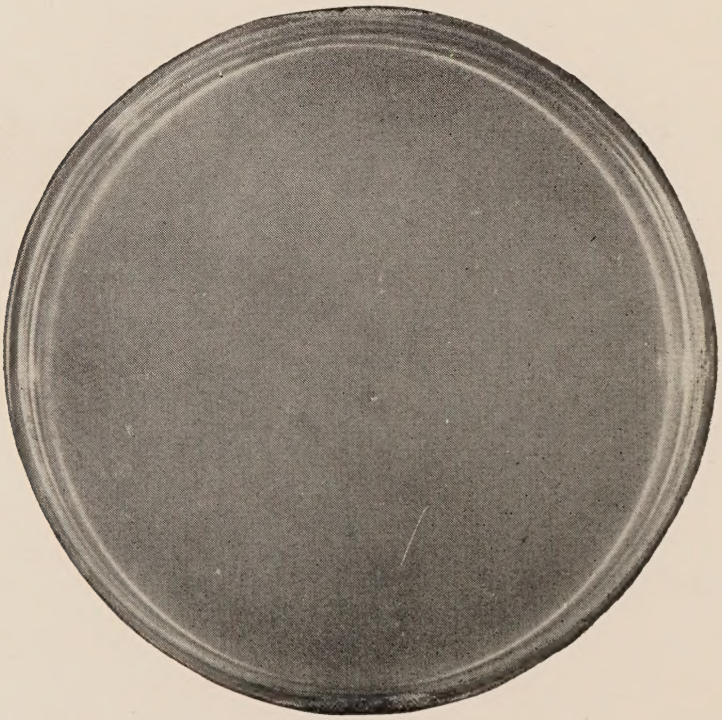

FIG. 2.—Photograph of similar agar plate prepared with vaccine material *four weeks* after GLYCERINATION.

VACCINATION

ITS NATURAL HISTORY AND PATHOLOGY

BEING THE MILROY LECTURES FOR 1898

DELIVERED BEFORE THE ROYAL COLLEGE OF PHYSICIANS OF LONDON

BY

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CHAPTER I

INTRODUCTION

JUST one hundred years ago, in the summer of the year 1798, there was published a treatise, the appearance of which marked a never-to-be-forgotten event in the history of scientific medicine. This truly epoch-making publication was entitled "An Inquiry into the Causes and Effects of the Variolæ Vaccinæ, a disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of the Cow-pox." It was the work of Edward Jenner, a country medical practitioner, living at Berkeley in the vale of Gloucester.

But in order adequately to appreciate the far-reaching importance of this work, first issued in the comparatively small compass of a quarto pamphlet of seventy-five pages, it is necessary for us, if possible, to transport ourselves in thought backwards for over a century, in order to realise approximately the truly terrible and far-reaching nature of the scourge, the only efficient preventative of which was first introduced to

the human race through the publication of Jenner's *Inquiry*.

It would be difficult to give a more vivid illustration of the state of affairs previously existing than is afforded by Macaulay's oft-quoted passage, in which reference is made to the death in 1694 of Queen Mary, wife of William III. He writes—

"That disease, over which science has since achieved a succession of glorious and beneficent victories, was then the most terrible of all the ministers of death. The havoc of the plague had been far more rapid; but the plague had visited our shores only once or twice within living memory; and the smallpox was always present, filling the churchyards with corpses, tormenting with constant fears all whom it had not yet stricken, leaving on those whose lives it spared the hideous traces of its power, turning the babe into a changeling at which the mother shuddered, and making the eyes and cheeks of the betrothed maiden objects of horror to her lover."

The ever-present danger, and the lively apprehensions engendered through recognition of the slight chance of any one evading the onslaught of the scourge between childhood and maturity, is also brought out somewhat forcibly in an extract from the "Pages from a Private Diary," in the January number of the *Cornhill Magazine* for the present year. It runs as follows—"I came on a curious passage in a letter of Mrs. Waller's to her banished son, about the marriage of his daughter. She wishes to know what dowry he is prepared to give. 'I am not in haste to marry her, she is young enough to

stay, but the danger is if she should catch the smallpox, or her beauty should change, it would be a great loss to her.' Everybody," adds the writer, "is familiar with the frequent references to smallpox in the letters and memoirs of the seventeenth century. Pepys is full of it; but I have never met with a passage that brings so keenly home to me the nearness of the risk."

In this connection also may be mentioned a record of an epidemic of smallpox in the small town of Ware in 1722. The population at the commencement of the epidemic was 2515, of whom 1601 are stated to have had smallpox previously, the remaining 914 being therefore presumably susceptible to the disease. During the course of the epidemic, of these 914 persons 612 were attacked, of whom 72 died. The remaining 302 persons who escaped attack are spoken of in the record in quaintly dogmatic fashion as "to have the smallpox."

We possess also a record of an epidemic which occurred at Warrington in 1773, where out of 473 deaths from all causes during the year, among a population estimated at 8000, no less than 211 were attributed to smallpox. And from a report by Haygarth of Chester, we learn, as in the case of Ware, how small a proportion of the population in these places had not suffered from smallpox at one time or another. The actual number of persons attacked at Chester in 1774 was 1202, of whom 202 died, the total population of the town being at that time 14,713. But as the result of an investigation carried out at the commencement of the following year, it becomes fairly certain that, previous

to the outbreak in 1774, there were in Chester of the whole population only 2262, or 15 per cent., who had not already had the disease. Consequently, of the remaining susceptible portion of the inhabitants more than half were attacked before the end of the year. These epidemics at Chester and Warrington further illustrate a marked characteristic of smallpox in the eighteenth century which is also noticeable in the records of preceding periods in which the age of those attacked is given, namely, the large proportion of the deaths contributed by children under ten years old. In the Warrington outbreak of 1773, and also in that which appeared in Chester in the following year, not a single death occurred in persons above this age, while of the 202 deaths at Chester, no less than a quarter of the total number were accounted for by infants of less than twelve months. Other instances of a like condition of affairs, noted in the Final Report of the Royal Commission on Vaccination, refer to Kilmarnock, where out of 622 deaths, occurring between 1728 and 1763, of which, with nine exceptions, the ages are recorded, only seven were of those above ten years, and to records obtained from the burial registers of the graveyards of St. Cuthbert's, Canongate, and Buccleuch Street, Edinburgh, from which we learn that, during the years 1764-83, of every thousand deaths from smallpox at all ages, no less than 993 were of children below ten years of age.

In considering the question of ratio of deaths from smallpox to the living population we are beset with several difficulties. In the first place, as McVail points

out, in no single epidemic in London in former centuries was it possible to see smallpox at its worst, since epidemics followed one another at such rapid intervals that of necessity the material on which at any one period their energy could exert itself was extremely limited. "At any given date the great bulk of the people had already had smallpox, and it was only among a fraction that there was room for the disease to spread. Yet it was among such fractions of the population that smallpox every few years slew its thousands in London alone." If we desire to obtain an illustration of the full effects of an invasion of smallpox, when not thus hampered by the existence of very general immunity, the result of former epidemics, we must turn our attention to some particular locality which, owing to its isolated position, has previously escaped invasion. In illustration of this point we may take the case of Iceland, where, during 1707-1709, out of a total population of 50,000, no less than 18,000 persons died of this disease.

Concerning the regions whence smallpox was originally derived, and the routes along which it subsequently spread, comparatively little accurate information is available. That the disease is of great antiquity in China and India is generally admitted, but it is fairly certain that it was not indigenous in Europe, where it appears to have been unknown prior to the end of the sixth century A.D., when the disease not improbably extended westwards from Arabia, it having broken out in a virulent form in the Abyssinian army of Abraha, which was besieging Mecca about 569 A.D. Hippocrates and Celsus were

apparently ignorant of its characteristics, and the same is probably true of Galen, although the Arabian physician, Rhazes, quotes three passages from Galen's works "κατὰ γένος," "On Pulses," and "On the Use of the Members," which he considers to prove that the older observer was not without knowledge of the disease.

Rhazes, in his work, quotes from the Pandects of Ahron of Alexandria, who flourished in the time of the Emperor Heraclius (610-641 A.D.), passages in which smallpox is undoubtedly referred to. Another writer, almost a contemporary of his own, also mentioned by Rhazes as having written a description of the disease, is Messua the Elder, who died in 857 A.D. The fact that at these dates smallpox was not of recent introduction is evidenced by the circumstance that no note to this effect is made by any of these writers, as otherwise might be expected to be the case.

To Gregory of Tours, who wrote in 581 A.D., we owe what is possibly a description of the first outbreak of smallpox on the continent of Europe, since in his description, "De lue quæ cum dessicis fuit," he definitely differentiates the disease in question from the plague with which it was in early days frequently confounded.

It was, in all probability, an extension to France and Italy of the epidemic described by Gregory, which has been handed down by the writings of Marius, Bishop of Averche, who, in the course of his description, makes the statement that "this year (570) a violent fever with flux of the bowels and variola affected both Italy and France."

Concerning this statement it is of interest to note that we have here what may be regarded as the earliest employment of the term "variola" in its modern sense. In his *Life* of Jenner, Baron states, however, that "The first recorded case of smallpox or variola under that peculiar and now appropriate name is probably that of Elfrida, daughter of our English Alfred and wife of Baldwin the Bald, Earl of Flanders: date of her illness is generally fixed as 907 A.D.; she recovered." The term "variola" has been stated to represent the diminutive form of the late Latin *varus*, a pimple, or, on the other hand, it may be derived from *varius*, meaning "spotted." By some authorities it is believed to have been first used in its modern sense by Constantinus Africanus in the eleventh century. The Anglo-Saxon equivalent of variola is found in the word *pocca*, meaning a bag or sack, which has given rise to the modern *pock*. The first mention of the term pocca would appear to be in a tenth century Leech-Book of the physician Bald; and subsequently it is employed in the *Chronicon Bertinianum* (1440 A.D.), wherein is set out an account of the illness of Baldwin, son of the Earl of Flanders, who died of smallpox in 961, the disease being described as "variolas sive poccas." Previously to the date of this and certain earlier writings, of which mention has been made, the word variola had apparently been used somewhat indefinitely as descriptive of any eruptive disease, inclusive of the plague. This was no doubt in part due to the defects of diagnosis, the study of medicine being at so low an ebb during the Middle Ages. In a reverse

manner the Anglo-Saxon equivalent *pocca* or *pock* has, since the appearance of syphilis in Europe, become restricted in its meaning, so as to designate the eruption peculiar to this disease alone. For this reason there gradually came about the use of the distinctive term *small*-pox, or its French equivalent *petite vérole*, as opposed to the *large* pock, or *great* pock, now exclusively reserved for syphilis.

To Rhazes himself, who flourished about the year 900 A.D., and who held the post of physician at the hospital at Bagdad, we owe what is really the first scientific account of the symptoms of smallpox, although he appears to have confounded the disease with measles. The treatise of Rhazes was printed in the original Arabic, together with a translation into Latin by Channing, in 1766, but it was not until 1847 that, under the auspices of the Sydenham Society, Greenhill published his English translation of this remarkable work. The descriptions of the various forms in which smallpox may present itself given by Rhazes are clear and definite, and the treatment advocated by him is in many respects in harmony with that which is now considered most appropriate. In support of this statement, it may be worth while to quote certain passages from his *Treatise on the Smallpox and Measles*—

"As to the moderns, although they have certainly made some mention of the treatment of the smallpox (but without much accuracy and distinctness), yet there is not one of them who has mentioned the cause of the existence of the disease, and how it comes to pass that

hardly any one escapes it, or who has disposed the modes of treatment in their right places.

. . . . . .

"Now the smallpox arises when the blood putrefies and ferments, so that the superfluous vapours are thrown out of it, and it is changed from the blood of infants, which is like must, into the blood of young men, which is like wine perfectly ripened; and the smallpox itself may be compared to the fermentation and the hissing noise which takes place in must at that time. And this is the reason why children, especially males, rarely escape being seized with this disease.

. . . . . .

"The smallpox arises from a fermentation that takes place in the blood, when it is undergoing the change from that of an infant to that of a young man; and it is accompanied by great heat and a disagreeable odour; and this is when the patient is of a hot temperament. The disease sometimes happens twice or even three times to the same individual, but generally once, especially to males, for the blood of infants must necessarily undergo this change.

. . . . . .

"I am now to mention the seasons of the year in which the smallpox is most prevalent: which are, the latter end of the autumn and the beginning of the spring; and when in the summer there are great and frequent rains with continued south winds, and when the winter is warm and the winds southerly.

. . . . . .

"The eruption of the smallpox is preceded by a continued fever, pain in the back, itching in the nose, and terrors in sleep. These are the more peculiar symptoms of its approach, especially a pain in the back, with fever; then also a pricking, which the patient feels all over his body; a fulness of the face, which at times goes and comes; an inflamed colour, and vehement redness in both the cheeks; a redness of both the eyes; a heaviness of the whole body; great uneasiness, the symptoms of which are stretching and yawning; a pain in the throat and chest, with a slight difficulty in breathing, and cough; a dryness of the mouth, thick spittle, and hoarseness of the voice; pain and heaviness of the head.

. . . . . .

"When, therefore, you see these symptoms, or some of the worst of them (such as the pain of the back, and the terrors in sleep, with the continued fever), then you may be assured that the eruption . . . in the patient is nigh at hand.

. . . . . .

"Ibn Másawaih says—'When there is with a continued fever, a redness of the eyes and face, much heaviness of the body and head, itching of the nostrils, sneezing, and a pricking pain all over the body, these are signs of the eruption of the smallpox. Your first care should be directed to the eye, for which you should use a collyrium made of sumach and rose-water, in order to prevent any pustules from coming out in it.

. . . . . .

"The eruption of the smallpox . . . is accelerated by well wrapping the patient up in clothes, and rubbing his body, by keeping him in a room not very cold, and by sipping cold water, a little at a time, especially when the burning heat is very great; for cold water, when it is sipped a little at a time, provokes sweat, and assists the protrusion of the superfluous humours to the surface of the body.

. . . . . .

"Extinguishing remedies are to be used when you see that, as soon as any of the pustules come out and appear in the skin, the patient finds himself easier after it, and his pulse and breathing are relieved in proportion; but if you see that the eruption and appearance of the pustules goes on slowly and with difficulty, you must in this case avoid all very extinguishing medicines, for to use them would be acting contrary to Nature, and hindering her from throwing out the superfluous humours upon the skin.

. . . . . .

"As soon as the symptoms of the smallpox appear, we must take especial care of the eyes, then of the throat, and afterwards of the nose, ears, and joints, in the way I am about to describe. And besides these parts, sometimes it will be necessary for us to extend our care to the soles of the feet, and the palms of the hands; for occasionally violent pains arise in these parts from the eruption of the smallpox in them being difficult on account of the hardness of the skin.

. . . . . .

"If a severe pain arises in the soles of the feet, then take care to anoint them with tepid oil, and foment them with hot water and cotton: . . . for these and the like things soften and relax the skin and thus facilitate the eruption of the pustules, and lessen the pain.

. . . . . .

"All those pustules that are very large should be pricked; and the fluid that drops from them be soaked up with a soft clean rag in which there is nothing that may hurt or excoriate the patient.

. . . . . .

"When the desiccation of the pustules is effected, and scabs and dry eschars still remain upon the body, examine them well, and upon those that are thin and perfectly dried up, and under which there is no moisture, drop warm oil of sesamum every now and then, until they are softened and fall off.

. . . . . .

" . . . and in order to efface the pock-holes, and render them even with the surface of the body, let the patient endeavour to grow fat and fleshy, and use the bath frequently, and have his body well rubbed."

It appears certain that during the time of the Crusades smallpox spread in a manner that has not unfrequently been witnessed subsequently under similar circumstances of war prevalence; the numbers of those dying from the scourge during this period, as far as can be learned from the indefinite accounts now available, having apparently been extraordinarily great. There is reason also for the

supposition that it was during the epidemic following the Crusades that smallpox houses were first erected. There is some authority for thinking that smallpox first found its way into England in the year 1241-42, although it may be stated that Creighton considers that there is no trustworthy evidence of its existence in England earlier than the sixteenth century. Towards the close of the thirteenth century the disease was referred to by Gilbertus Anglicanus in his *Compendium Medicinæ*, which is probably the earliest medical work produced in England. But although he gives an account of the symptoms of smallpox and measles, he was not, so far as can be judged, himself acquainted with the disease, and he did no more than slavishly follow the early accounts of the Arabian physicians. The same statement applies also to his successor, John of Gaddesden, physician to Edward II., whose work, *Rosa Anglica*, appeared about the year 1320. He gives a description of an attack of smallpox from which the King's son[1] suffered. For this, Gaddesden treated him by wrapping him in a red cloth, and by entirely enclosing the bed in hangings of a red colour, with the effect, he says, that no pitting of the skin resulted from the attack. This method of treatment, however, did not originate with Gaddesden, having been suggested by former writers of the Arabian schools, to whom reference has already been made. Baron says of Gaddesden that "his only dogma worth recording with regard to smallpox is 'aliquando variolæ

[1] Dr. Norman Moore supposes the "King's son" to have been Thomas of Brotherton.

bis hominem invadunt,' " and Sir Thomas Watson somewhat unreasonably opines him to have been a sorry knave.

The use of red hangings and wraps was devised by its earliest advocates with the object of drawing the peccant humours of the body to the surface, while puncture of the pustules by means of a golden needle, or even a thorn, was relied on for the prevention of pitting. That Gaddesden knew of this latter method is obvious from his writings, but he does not make any mention of having practised it on the King's son. For this reason Creighton expresses the opinion that the disease, concerning which the old physician says that he obtained a good cure, and *sine vestigiis*, was in all probability something of a much less formidable nature than variola.

Creighton further asserts that it is not until the year 1514 that a definite reference to smallpox is to be found, this being in a letter in which mention is made of an illness from which the king, Henry VIII., had recently recovered, "*nommée la petitte verolle.*" The English equivalent of this term, however, is shown by the researches of Dr. Norman Moore to have been in use at a considerably earlier date. The work cited by Dr. Norman Moore is a manuscript copy of the *Breviarium Bartholomæi*, compiled by John Mirfield, a canon regular of St. Austin in the priory of St. Bartholomew in West Smithfield, and written about 1387 for the hospital of St. John the Baptist, attached to the Abbey of Abingdon. On folio 43*a* of the

manuscript, which is now in the library of Pembroke College, Oxford, after the heading "De variolis et morbilis" the words, "*i.e.* smal pockes" are written.

During the sixteenth century, references to attacks by the disease occur with gradually increasing frequency. It would appear, however, from the perusal of such records as are available, that the disease was not for the most part of extremely virulent type, and also that its power of infectivity had not as yet been sufficiently appreciated to cause it to be feared, at any rate, as was the plague, for instance. In support of this assertion may be mentioned a death-bed statement by a certain Master Richard Allington, recorded by John Stow as having been made in the presence of the Master of the Rolls and four other eminent lawyers, in which the following passage occurs:—

"Maisters, seinge that I muste nedes die, which I assure you I nevar thought wolde have cum to passe by this dessease, consyderinge it is but the small pockes. . . ."

In 1604, the classification of deaths in London by the Company of Parish Clerks was commenced, although their annual and weekly records were not regularly printed until a quarter of a century later. During this interval smallpox made rapid progress in England, the literature of this and succeeding periods now teeming with allusions to the "cruel and impartial sickness." Dr. Gee, in an address to the Abernethian Society, quotes numerous instances:—"Even the poets could not avoid the disgusting theme. If they wished to

bewail the death of a friend, in all probability he died of the smallpox. Dryden wrote elegies upon two of its victims. The first was Lord Hastings, who died, 1649, at the age of nineteen. Mrs. Anne Kelligrew died of variola in 1685 at the age of twenty-five, and she attained the honour of being celebrated by the same poet in much nobler verses. Mrs. Katherine Phillips, 'the Matchless Orinda,' died of smallpox in 1664 at the age of thirty-three; she was lamented by Cowley. In 1675 Oldham devoted an ode of extraordinary length to the memory of his friend, Mr. Charles Morwent, who was carried off by smallpox. Writers of a satirical turn condoled with ladies upon the sad loss of beauty which ensued when they escaped with life from the dreadful pest. Verses were written, 'Upon a Gentlewoman, whose nose was pitted with the Smallpox,' and so on."

It was during the epidemic of 1694 that Queen Mary, the wife of William III., who was then only thirty-three years old, became attacked with what eventually turned out to be hæmorrhagic smallpox. To this attack she succumbed on the eighth (ninth?) day of the disease. The number of deaths from smallpox recorded in London alone during this year was not far short of 1700, but this figure had been exceeded at least seven times during the previous half century, most markedly so in 1681, in which year no less than 2982 deaths occurred in London from this disease.

From 1695 onwards for a series of years, the mor-

tality from smallpox underwent considerable diminution; but in 1710 a terrible epidemic accounted for no less than 3138 deaths in London, and we learn, on the authority of Blomefield, that the disease also cut off "great numbers in Norwich." The year 1714 was also marked by epidemic prevalence of smallpox in London, which resulted in an almost equal number of deaths; while the record of 1710 was even exceeded in 1719. Beyond this period it is needless to extend our review, seeing that the history of smallpox prevalence now becomes intimately bound up with the history of the spread of certain prophylactic measures, the effect of each of which on the disorder will require separate consideration.

Aitken calls attention to the fact that since the date of the first accounts by the Arabian physicians of the ravages of smallpox in Mecca, the history of this disease may be arranged in three great stages, each of which is characterised by remarkable epochs. The first of these periods is marked by an improvement in the treatment of smallpox, the merit of this revolution in medical practice being due to Sydenham. In few diseases, indeed, has medical opinion undergone in the course of years a more obviously beneficial change. The second stage is marked by the discovery of the singular phenomenon that the virulence of the poison of smallpox is greatly mitigated by introducing or engrafting the disease into the system through the cutaneous tissues, thereby causing the transference of the disease from one person to another by inoculation.

To Lady Mary Wortley Montagu in 1721 we owe the introduction of the practice of inoculation into this country, a deed which must be considered as one of no little bravery, when measured by the knowledge possessed by physicians in those days. The third great era is characterised by the remarkable discovery, which has rendered the name of Jenner immortal, namely, the modifying and protecting influence of vaccination.

With the therapeutic treatment of smallpox we are not on the present occasion concerned. On the other hand, the practice of inoculation, although prohibited by law since the passing of the first Vaccination Act, 1840, is yet deserving of some attention. Having dealt with this matter, we shall be in a position more fitly to discuss the question of the discovery of vaccination, and of the evidence on which its employment was originally based.

There will remain for our consideration a review of such accretions to our knowledge of the subject of vaccination, more particularly from the standpoint of pathology, as the progress of scientific research during the century since Jenner wrote has rendered available.

To marshal these in order, to try to indicate their relative importance, and to direct attention to their bearing on the necessity for certain changes in the regulation and practice of vaccination, as these obtain at the present time, is the chief aim which I have had in view in the preparation of these lectures.

Inoculation

Recognition in former times of the certainty that almost every one would suffer attack by smallpox during some period of his or her life, and the lively horror inspired by the disease, by reason not only of the high fatality inseparable from it, but also of the maiming and disfiguring effects on a large proportion of those who survived its invasion, naturally turned men's minds to the question as to whether it were not possible to devise some method for mitigating the virulence of this disorder.

Whence, however, was originally derived, or by whom, accidentally or otherwise, was discovered the artificial method of communicating smallpox, which came to be known by the term "inoculation," it is at the present day impossible to determine.

According to tradition, the process of inoculation has been in use among the Brahmins in India for centuries, and a similar claim has been made on behalf of the Chinese.

There can be little doubt that in China a method of artificially communicating smallpox was in vogue long ages apparently before the disease had obtained recognition in Europe; but the process was essentially different from inoculation properly so called, consisting as it did of inserting smallpox crusts into the nostril of the patient, whereby the disease was communicated by way of the respiratory tract. Coming to comparatively recent times, however, we have definite evidence that inoculation was practised in Turkey early in the eighteenth century.

In the twenty-ninth volume of the *Philosophical Transactions of the Royal Society* may be found references to the process, as observed in 1713 by Dr. Emanuel Timoni of Athens, and in 1716 by M. Pylarini, then Venetian Consul at Smyrna. But it would seem that their descriptions made little, if any, impression at the time, and, although published in the records of the Royal Society, passed almost at once into oblivion.

The actual introduction of inoculation into England was, as just stated, brought about through the instrumentality of Lady Mary Wortley Montagu, who, apart from her social status as wife of the English Ambassador at the Ottoman Court, has achieved for herself lasting recognition in literature, by reason of her attainments in the gentle art of letter-writing.

Indeed, Professor Rayleigh, in a recent lecture at the Royal Institution, gave it as his opinion that in this respect she should be placed second only to Madame Sevigné. Writing from Adrianople, in 1718, she says: "The smallpox, so fatal and so general amongst us, is here entirely harmless by the invention of *engrafting*, which is the term they give it. Every year thousands undergo the operation, and the French Ambassador says, pleasantly, that they take the smallpox here by way of diversion, as they take the waters in other countries. There is no example of any one who has died in it, and you may believe I am well satisfied of the safety of this experiment, since I intend to try it on my dear little son. I am patriot enough to take pains to bring this useful invention into fashion in

England." Lady Montagu had indeed the courage of her opinion, and the first person inoculated in England was her daughter. This first operation, by Dr. Maitland, in April 1721, was shortly followed by the inoculation of a child of her family physician, Dr. Keith. Next an experiment on a larger scale was carried out on certain condemned felons, who were offered the alternative of submitting to the operation. No ill result having been witnessed thus far, King George I., who had been much interested in the matter, was himself inoculated, as also were several members of his family. Apparently, however, the method did not generally commend itself to public taste; moreover, it was discovered that, contrary to anticipation, persons inoculated with smallpox became themselves centres of infection; and perhaps for the reason that no serious attempt was made at that time to diffuse information on the subject, it was not until thirty years, or more, subsequent to its introduction into this country, that inoculation as a prophylactic against smallpox became practised to any considerable extent.

In his *Synopsis Medicinæ*, published in 1761, Dr. John Allen, a Fellow of the Royal Society, gives the following curious directions for the inoculation of the smallpox—"First, a little quantity of purulent Matter must be procured, about 5 or 6 drops taken from the Pustles of some young Person, who has the benign Small-Pox of the distinct Kind, which must be brought to the place where the operation is to be performed in a

Small Vial or in a little Pill-Box; and when all Things are prepared, the Surgeon is to make two small Wounds, one on the Arm, and the other on the Leg, of the Opposite Side; and the aforesaid variolous Matter is to be applied to the Wounds on Dossils of Lint, and a Bandage to be made over it. There is no occasion to make the incisions deeper than just through the Skin, nor any longer than a Barley-Corn. After twenty-four Hours the Dossils may be removed, and the Ulcers dressed with Diachylum Plaister or Cole-Wart leaves once or twice a day, according as more or less Matter is discharged. This being done, the Distemper will gradually be produced; and it is usual for the following Symptoms to arise. About the eighth day after the Operation some Pustles begin to appear not unlike to those that are commonly seen in the distinct kinds, a little Fever having preceded the Eruption, and the other usual Symptoms but more mild and gentle. As to the places where the Incisions are made the fourth Day they grow red and are inflamed, on the sixth they tend to Suppuration, and discharge an ichorous Pus. Indeed, about the time of the Eruption of the Pustles, whilst the little Fever lasts, the Ulcers discharge somewhat less; but after the Eruption even until the Decline of the Pustles, the Pus increases daily, and after that it again gradually diminishes; so that commonly in five weeks' time the Ulcers are quite dried up. By how much the greater the discharge of purulent Matter is by these artificial Ulcers so much the milder will the Distemper be. In the general it is observable that the Small-Pox

procured by Inoculation are of the distinct Kind, for the most part void of Danger, that the Pustles are few in number, and pit very little. Very often however there follow after them Tubercles and Abscesses, some indeed but slight ones but sometimes very malignant ones, and exceedingly difficult of Cure.

"To prepare the Body for Inoculation a great many talk much of Bleeding, of giving Emeticks, and Cantharticks. But in truth, if the Person to be Inoculated has Youth on his side, is of a good Habit of Body, and in Perfect Health, none of these things seem to be necessary. It is requisite only, that he take care to observe a temperate Regimen for some time before the Operation. When the Operation is performed, the Person need not be kept under any strict Confinement, but may live pretty much after his accustomed Manner; excepting that it will be prudent to forbid him the eating any Flesh-Meat. When the Small-Pox are come out, the same Regimen is to be observed, as is usual in the distinct Kind, got in the common way; and if there be occasion for anything at all of Medicine, these procured by Art require the same Method of Treatment, as the benign Discrete Small-Pox in the natural way."

The subsequent extensive diffusion of the practice of inoculation in this country was undoubtedly due in large measure to the labours of two brothers named Sutton, and also of Adams and of Dimsdale, of whom the last named had a barony conferred upon him in commemoration of his having successfully inoculated

the Empress Catherine of Russia. For his services he also received a fee of £10,000, which has been estimated as equal to at least three times that sum at the present day, and £2000 as an allowance for travelling expenses. He was also appointed Councillor of State and Physician to Her Imperial Majesty, with an annuity of £500, which was punctually paid him till his death in the early years of the present century.

The Suttons—two brothers, Robert and Daniel, who lived at Bury St. Edmunds, Suffolk, and Ingatestone in Essex, respectively—although not medical men, introduced or rather revived the greatly improved method of treating the patients under their charge. They were extraordinarily successful in the results which they obtained, and indeed claim to have inoculated no less than 20,000 persons without losing one as the direct result of the operation. Acting on the instructions originally laid down by Sydenham for the treatment of ordinary smallpox — the form of treatment to which, by the way, his contemporaries obstinately refused their adherence — the Suttons adopted and caused their patients strictly to observe what was known as "the cooling treatment," a method much akin to that which appears to have been pursued with good results by the Turkish operators from whom Lady Montagu first derived the knowledge of the process of "engrafting." In the hands of Daniel Sutton, who became specially famous for his successful inoculations, "the great secret of his success seems to have consisted in his making one puncture only; exposing his patients much and often

to a cool atmosphere; supplying them freely with refrigerant drinks; and restricting them to a spare diet."

By attention to the mode of life and general treatment of persons undergoing the process, together with careful selection of the sources (preferably the primary vesicle), from which the virus was obtained, Adams indeed succeeded in gradually evolving a strain of virus of such tenuity as to produce in a considerable majority of instances nothing beyond a single vesicle at the original site of puncture. The general eruption, typical of the disease as contracted in the ordinary manner, was in these cases either entirely absent or represented by, at most, a very few secondary vesicles only. Although, doubtless, the extremely mild form of the disease, thus artificially produced, was none the less contagious, the visible effect produced so closely resembled the results then beginning to be known as following on the Jennerian process of vaccination, that numbers of Adams' patients could hardly be persuaded that he had not, contrary to their desire, intentionally vaccinated rather than variolated them.

There has always been considerable controversy as to the efficacy of the process of inoculation in restraining the ravages of smallpox, the matter being rendered the more difficult since it is almost impossible to arrive with any accuracy at what may be thought of as the average death-rate of the natural disease, which in pre-inoculation times is known to have varied greatly in different epidemics. If, as has been frequently asserted,

the average mortality in natural smallpox may be taken as being one in five or six, and, on the supposition that in the majority of cases, at any rate, inoculation prevented subsequent invasion by smallpox, then it is quite evident that the process was of the utmost value to the persons inoculated.

The statement of the Suttons, already mentioned, that inoculation in their hands was unattended with fatal results, is perhaps exaggerated, as may also be the declaration of Baron Dimsdale, that in a series of 15,000 cases no death had occurred. Nevertheless, the records of the Smallpox Hospital for a series of years show that we shall not greatly err, and shall indeed be probably within the mark, if we accept Dr. Gregory's estimate of 1 death in 500 inoculations as approximately correct. There is, however, perhaps ground for suspicion that inoculations performed in the face of epidemic smallpox were more prone to be fatal than were inoculations undertaken when the disease was not prevalent. On the other hand, we must bear in mind that the general diffusion of inoculation brought under the influence of the disease, although possibly in a mild form, large numbers of persons who otherwise might probably have escaped infection.

It is, I think, hardly possible better to sum up the matter than in the words of Sir Thomas Watson: "The advantages of the practice of inoculation to the individual, supposing him doomed to have smallpox, were great and obvious; to the community at large they

were very doubtful. It gave the undoomed individual, for certain, an ugly disease, which was comparatively free from danger, in exchange for the chances, on the one hand, of contracting a very hazardous form, and, on the other, of escaping altogether from any form of variola. We need not inquire which is the most eligible branch of this alternative; we know which was by most men actually chosen. But the practice of inoculation, by carrying the virus and the disease into every village throughout the length and breadth of the land, filled the country with contagion; ensured the disease to all who were subjected to the operation, and diminished to all who were not the chances of escaping it. No doubt the distemper was produced artificially in many more persons than would have caught it naturally, had inoculation never been thought of. So that while the relative mortality, the percentage of death from smallpox, was lessened by this practice the absolute mortality was fearfully increased. Such at least is the judgment expressed by most who had thought and written on the subject."

As there appear to be few, if any, records as to the fees obtained by inoculators, it may be of interest here to insert a copy of a small handbill, which is in the possession of Mr. Parson, the senior medical practitioner in Godalming, Surrey. This relates to the practice of inoculation, as carried out by a direct ancestor and predecessor, in the same practice, of Mr. Parson—

INOCULATION

MR. PARSON, Surgeon, at HASLEMERE, having provided a convenient Houſe (within a Mile of that Town) for the Reception of Patients, propoſes to inoculate from this Time to MAY next, at ten ſhillings and ſixpence each Perſon.

10th. DECEMBER, 1783.

PETERSFIELD, Printed by T. WILLMER.

VACCINATION

It was during Jenner's apprenticeship to Mr. Ludlow, a medical man practising at Sodbury, near Bristol, that his attention first became directed to a belief widely prevalent in Gloucestershire during the latter half of the eighteenth century, that those persons who in the course of their employment on dairy farms happened to contract cow-pox, were thereby protected from a subsequent attack of smallpox.

Although himself a native of Gloucestershire, he does not appear to have been aware of the local tradition, previously to joining Mr. Ludlow at Sodbury. It was while working here that his interest became aroused by a casual remark made by a young country-woman, who happened to come one day for advice,

and who on hearing mention made of smallpox, immediately volunteered the statement that she could not take that disease as she had had cow-pox.

On coming up to London in 1770, to finish his medical education, Jenner entered at St. George's Hospital, and became also a pupil of John Hunter, with whom he speedily became a great favourite. With Hunter he discussed the question of the protective power of cow-pox over smallpox, but the great anatomist does not appear to have attached much importance to the matter, although subsequently on several occasions he referred to it both publicly and in private. In 1773 Jenner returned to his native village of Berkeley, where he commenced to practise as a medical man. The impression which had been made on him in his student days as to the possibility of obtaining protection against smallpox still persisted, becoming even stronger as time went on. It would appear, indeed, to have been ever present in his mind, and at length he determined to put the matter to the test of direct experiment.

It was not, however, until 14th May 1796, which has been appropriately termed the birthday of vaccination, that he commenced actual experiment. On this day he vaccinated James Phipps, a healthy boy about eight years old, by inserting into two superficial incisions on his arms, matter taken from a vesicle on the hand of Sarah Nelmes, a dairymaid, who had been infected while milking her master's cows. The result of this experiment, the details of which are so well

known as to need no mention here, must have afforded Jenner the most lively satisfaction, conforming as it did to what, up to that time, had been with him a pious opinion only. "But the most agitating part of the trial still remained to be performed. It was needful to ascertain whether he (Phipps), was secure from the contagion of smallpox. This point, so full of anxiety to Dr. Jenner, was fairly put to issue on the first of the following July." Variolous matter, taken directly from a pustule, was carefully inserted by several incisions, but no result followed.

Having succeeded in communicating cow-pox from one human being to another by implantation of the virus in the skin, the next step was obviously to attempt its transference to yet another subject, and so on if possible through an indefinite series, in order to ascertain whether its protective power was hereby diminished. Through lack, apparently, of material, Jenner's work was interrupted until the spring of 1798, when a simultaneous outbreak of cow-pox and "grease" afforded him the further opportunity he desired.

A few months later he published his *Inquiry*, in which were set out the results of his observations. The appearance of this work was the signal for an extraordinary wave of enthusiasm, which spread so rapidly that, within a few years only of Jenner's announcement, a knowledge of his method of preventing smallpox had extended to almost every portion of the civilised world.

The experience of the past century, culminating in

the definite pronouncement in 1896 of the Royal Commission on Vaccination, having fully justified the principle of Jenner as to the prophylactic power of vaccination, it may be of interest to determine what light pathology is capable of throwing on the relationship which may be thought of as existing between variola and vaccinia.

Pathology of Vaccinia

Vaccinia is, in the human subject, a specific disorder characterised by the appearance of a local eruption passing through the stages of papule, vesicle, and pustule, associated with more or less constitutional disturbance.

These symptoms are produced indifferently, by the inoculation of lymph derived from vesicles similarly brought about in a previous case in the human being, or from the eruptive vesicles of a disease of bovine animals, called cow-pox. Such inoculation process, whichever way induced, is known as vaccination. This name was originally devised by Mr. Dunning of Plymouth Dock, inspired doubtless by the terminology of Jenner, who wrote of the disorder under the title of Variolæ Vaccinæ. In this manner Jenner gave expression to his belief that the malady commonly known as cow-pox was, in reality, nothing more nor less than smallpox of the cow.

But soon it was discovered that if there was such a malady as "smallpox of the cow," there was also a smallpox of the horse, which, under the name of "grease," was resorted to as a source of vaccine lymph.

Jenner, indeed, was undoubtedly of the opinion that cow-pox originated from the transmission, most probably by human agency, of infection from a previous case of horse-pox. At the time of publication of his *Inquiry*, he had not indeed been able to adduce any direct experimental evidence in support of his contention, although he had collected a number of observations which, in his opinion, afforded reasonable probability as to the correctness of his view.

Mr. Tanner, however, shortly after put the matter to the proof, and was successful in communicating the disease to the cow, by employing for its vaccination lymph taken from the heel of a horse suffering from "grease." The attempt resulted in the appearance of a perfect vaccine vesicle on the cow's teat, at the site of insertion of the lymph. Tanner states that: "From handling the cow's teats, I became infected myself and had two pustules on my hand, which brought on inflammation, and made me unwell for several days. The matter from the cow, and from my own hand, proved efficacious in infecting both human subjects and cattle."

Dr. Loy was the first to distinguish, in any satisfactory fashion, constitutional "grease" from a merely local affection, with which it was apt to be confounded; and thus he explained the failure on the part of many experimenters to transmit horse-pox to the cow.

Dr. Sacco of Milan, who, in the first instance, had expressed opinions adverse to Jenner's views, subsequently, in a letter written to him in 1803, withdrew

PLATE II.

CASUAL HORSE-POX.

George T——, stableman, *æt.* 30, vaccinated in infancy.
From a photograph taken 6th April 1898 (eighth day of disease).

them; his reasons for which may perhaps be best given in his own words—

"MILAN, *le* 25 *mars*, 1803.

"MONSIEUR—J'étais depuis longtemps occupé à faire des expériences sur le *grease* pour confirmer votre opinion sur l'origine de la Vaccine. Jusqu'au commencement de cette année je n'avais jamais pu rien obtenir. La lecture du petit livre de Mr. Loy m'encouragea à répéter une autre suite. L'hiver de cette année ne pouvait pas être plus abondante de grease à cause de la quantité de l'eau qu'il y avait, et par conséquence de la boue dans les chemins: ainsi presque tous les chevaux souffraient le grease. Mon domestique en fut attaqué au deux avant-bras par cinq boutons pansant un de mes chevaux qui avait le grease; il ne m'en averti que quand les boutons passaient en exsiccation; celui m'encouragea de plus à continuer mes tentatives. J'ai inoculé plusieurs enfans, plusieurs vaches avec le virus qui sortait du grease à différentes époques, mais toujours inutilement. Un cocher se présenta à l'hôpital pour se faire visiter d'une éruption qu'il avait sur les mains. On connait de suite que c'était vaccine prise en traitant les chevaux qu'effectivement il pansait. Il fut conduit à l'hôpital des enfans trouvés où on fit quelques inoculations: il vint le même jour chez moi, et je fis neuf inoculations sur autant d'enfans, et de plus j'ai inoculé les pis d'une vache. Trois de ces enfans ont contracté une éruption toute pareille à la Vaccine. La

vache n'a point pris. J'ai fait des autres inoculations avec la matière prise de ces enfans, et c'est déjà la quatrième génération que se reproduit avec la même effet comme le Vaccin. J'ai déjà inoculé plusieurs de ces individus avec la petite vérole, mais sans aucun effet. C'est donc bien sûr et consenté que le *grease est cause de la Vaccine*, et on pouvait bientôt changer dénomination en *équine*, ou en ce que vous croyez mieux. J'ai aussi enfin obtenu avec le virus de grease inoculé sur six autres enfans deux boutons tous semblables aux Vaccins. Je continue mes observations. Il y a tout pour s'assurer qu'enfin nous aurons du grease le virus pour se mettre à l'abri de la petite vérole sans passer aussi par *l'intermedium* de la vache. J'espère que cette nouvelle preuve pourra ôter les doutes qu'il y avaient encore sur l'origine de la Vaccine. Je publierai les résultats de ces expériences sur un code doctrinal de vaccination, auquel j'ajouterai une planche illuminé de grease. J'espère que vous aurez reçu les médailles par Mr. Woodville, à qui je me pris la liberté d'adresser le paquet pour vous le faire obtenir avec certitude. Je renouvellais mes remercîmens pour les livres que vous m'avez envoyés, avec les regrets aussi de n'avoir pas reçu aucune de vos lettres. Je comte à cette heure plus de 25 mille inoculations faites par moi seul. Je vous prie, mon très-estimable collègue, de me donner quelque nouvelle avec quelqu'autre enseignement sur cette matière, mais surtout tout honorez-moi de votre réponse.

Très humble serviteur,

(Sd.) Louis Sacco, *Med. Chirurg.*"

That a constitutional disease of the horse, characterised by a vesicular eruption, can be induced by the inoculation of this animal with the virus of cow-pox or vaccinia, has been shown experimentally by Chauveau, who injected vaccine lymph subcutaneously and also into the blood-vessels and lymphatics of colts. In nearly half the number of cases operated on, the injection of the lymph was followed by a generalised eruption which Chauveau called "horse-pox." In all probability Jenner was mistaken in his assumption that "grease," in the sense of horse-pox, was a necessary antecedent to cow-pox; but at the same time there can be little doubt that the two diseases are very closely allied, if indeed they be not identical.

That this is so is shown by the fact that numerous strains of vaccine lymph have, from time to time, been raised from the equine source—the protective power of which against smallpox we have reason to believe was equal to that exhibited by lymphs of undoubted bovine origin. As, however, it is obvious that cow-pox was the source of the lymph stocks first introduced into use by Jenner and his contemporaries, it will be of interest to study briefly the nature and clinical appearances of this disease as seen in the cow and also in man; whether accidentally contracted or intentionally inoculated.

Cow-pox in the Cow.—For a description of cow-pox in typical form, as it was known to Jenner and his contemporaries, it is necessary to consult the writings of the early part of the century, at which period the attention of the medical and scientific world had been specially

directed to this affection of cows by the teaching of the apostle of vaccination. Probably the most trustworthy accounts are those published by Bryce of Edinburgh, and later by Ceely; and it is from their statements that the following description of the malady is derived.

According to these observers, this affection, when once set agoing in a herd, tends to spread with considerable rapidity, the "matter" of the vesicles being carried by the milkers from one cow to another. It makes its appearance especially in the spring season, and is observed upon the udders and teats of the cows; at first in the form of small vesicles containing a limpid fluid. These vesicles are of a bluish or livid colour, and are surrounded with considerable erysipelatoid swelling and inflammation. If ruptured, the vesicles tend to become irregular about the edges, and unless care be then taken are apt to degenerate into foul and troublesome sores. During the course of the affection the cow is not unfrequently observed to be in bad health; the appetite is impaired, the temperature is above normal, and the secretion of milk may be considerably diminished. If the material from the vesicles on the udders or teats of the cows happen to come in contact with an abrasion of the skin of the milker's hand, such person is apt to become infected with the disease. When the ailment is communicated in this manner, it is termed *casual cow-pox* to distinguish it from that form which is intentionally propagated by inoculation, under which conditions the affection is less virulent than when communicated in the former way. Probably

the more severe form in which casual cow-pox usually appears is to some extent due to the situation of the resulting vesicles, and to the purulent nature of the secretion from the sores on the cows' teats or udders.

Casual Cow-pox in Man.—When cow-pox has been communicated to the milkers in the casual way, small inflamed spots appear in a few days upon the hands, more particularly about the joints and tips of the fingers. These spots quickly assume the appearance of small blisters, somewhat resembling those from burns, which go on increasing until they become large vesicles of a circular form, with a flat or rather a concave surface; their edges being considerably elevated above their centre. They have then acquired a somewhat bluish colour, and are found to contain a limpid fluid. After some days the parts around the base of these vesicles become considerably swollen, hard, and inflamed, and, as the affection advances, they may assume something of an erysipelatous appearance. Pain and some degree of swelling of the axillary glands now denote an absorption by way of the lymphatics, and, with the usual symptoms of fever, mark a constitutional affection which is sometimes so severe as to incapacitate the person from following his usual employment for some days. It does not appear, however, that a general eruption ever follows even on the smartest attack of casual cow-pox. After a few days the pain, inflammation, and hardness of the surrounding parts gradually abate; but the vesicles not infrequently ulcerate instead of becoming encrusted and drying up. These ulcerations, however,

gradually heal up in course of time without occasioning any lasting injury; and the constitutional affection, although severe, is always transient and unattended with danger: there is no case on record in which casual cow-pox is known to have been fatal.

Inoculated Cow-pox in Man.—In the cow-pox induced by inoculation the appearances which present themselves may differ considerably in some respects from those which have been described as occurring in the casual disease.

Thus about the third day after the insertion of the virus of cow-pox, either by puncture or by slight incision in the arm, a small inflamed spot may be observed at the point where the inoculation was performed.

Next day this spot appears still more florid, and on passing the point of the finger over it, a certain degree of hardness and swelling is readily perceptible.

By the fifth day a small pale vesicle occupies the spot where the inflammation began, and the affection begins to assume the characteristic appearance of cow-pox. The vesicle has now a milky-white colour without any inflammatory zone around it, it is evidently depressed in the centre, and its edges are considerably elevated.

For the next two days the vesicle increases in size and retains the same character, so that by the seventh day it has acquired very considerable magnitude; if the inoculation be performed by a puncture, it assumes a circular form, if done by an incision, an oblong form. But in both cases the margin is regular and well

defined, while the centre becoming still more depressed and the edges more turgid, the whole puts on an appearance which is very characteristic of this particular affection.

About the eighth day from the time of inoculation an inflammatory zone begins to appear round the base of the vesicle. This increases for two or perhaps three days more, by which time it may be two inches or longer in diameter, and of a bright red colour. At this period, also, the vesicle still retains its concave appearance; the crust in the centre has considerably increased in size, and begins to assume a dark or brownish colour.

About the eleventh day the vesicle has attained its greatest magnitude, and the surrounding inflammation begins to abate. The fluid in the vesicle, which before was thin and transparent, is now more viscid and slightly turbid. After this period the whole becomes quickly converted into a smooth, shining, and somewhat translucent dry crust of a dark brownish or red colour.

This crust, unless forcibly removed, will adhere for a week or more, and then fall off, leaving the skin beneath apparently sound, but livid for a time, and more or less permanently scarred.

In children little else than the above local process is usually noticeable, but in adults constitutional symptoms are apt to be somewhat severe. About the eighth day from the time of inoculation the glands in the axilla become a little swollen, and there is pain and stiffness

on moving the arm. Headache, shivering, a rapid pulse, and other febrile symptoms present themselves, and these may persist for a period varying from a few hours to two or more days.

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CHAPTER II

THE RELATIONSHIP OF VARIOLA AND VACCINIA

ALTHOUGH it is clear from Jenner's writings that he believed vaccinia to be nothing more nor less than "smallpox of the cow," his theory appears not to have received general support even in his own day, and from that time onwards the value of the practice of vaccination has by some been impugned on the plea that inoculation of one disease—"cow-pox"—could not be expected to exert any really protective influence against the ravages of smallpox, a disease supposed by them to be of totally different origin. And if the thesis of essential difference between these maladies were capable of demonstration, no doubt the objection would be of considerable weight. For there exists but little well-authenticated evidence that the living virus of one disease is capable, when inoculated into an animal, of affording protection against the effects of inoculation of the virus of another and totally different disease, although, no doubt, when two different viruses are inoculated at nearly one and the same time, the incubation period of one or other of them may be modified as to duration.

In his first paper, Jenner, as has been said, advanced

the thesis that smallpox and cow-pox are identical; but even at the present day controversy wages hotly around this question, and it still awaits a definite solution. There can, however, be no objection to our speaking of vaccinia as "one with smallpox," since it matters little from our present point of view whether smallpox, on its transference from man to the bovine animal, becomes actually transformed, or, as some would maintain, merely modified. During the long period which has now elapsed since the introduction of vaccination, many observers have set themselves the task of attempting, by experimental methods, to solve the problem of the true relationship of variola to vaccinia. These attempts have all been directed to the possibility of giving rise to cow-pox by the introduction in one or another manner of the virus of smallpox into the system of the bovine animal. In the great majority of such attempts, which are vastly more numerous than is generally supposed, the results have been entirely negative, although so numerous have been the experimenters, who from time to time have attacked the problem, that the total number of instances in which an apparently successful result has been obtained is now considerable.

So far as I am aware, the first recorded experiments are those of Gassner of Gunzburg, in 1801, who succeeded, after no less than ten fruitless attempts, in directly inoculating a cow with smallpox virus. The lymph thus obtained was employed for the vaccination of four children, from whom other seventeen were subsequently vaccinated. None of these exhibited any

signs of smallpox. Viborg of Copenhagen, in 1807, is stated to have also been successful in variolating the cow. Basil Thiele of Kasan twice, in 1836 and 1838 respectively, succeeded in inoculating the cow with smallpox matter, and in vaccinating children with the lymph thus obtained. In the light of recent work on this subject, his conclusions are of much interest. As stated by himself, they are: "(1) The so-called vaccine disease is not an eruptive disease peculiar to the cow, but it is produced in it by the transmission of human smallpox to it, and the man and not the cow, as has hitherto been thought, is the source of the disease. (2) The mild disease thus caused in the cow can, by direct transmission from the cow to man, produce in him as mild a disease, which gives protection against the natural smallpox." Thiele also described a method by which he claimed to have brought about such modification of smallpox virus as to render benign its action on the human subject, without the intermediate stage of implantation on the tissues of the cow. This altered potency of the material was brought about, according to his statement, by keeping the smallpox lymph for a period of ten days between two slips of glass, after which milk was added to it, and the resulting mixture employed for the vaccination of children. The same process was carried through with the lymph obtained from the first and following series of children vaccinated, until after ten generations addition of milk was found to be no longer necessary.

Seeing that at this date (1836-38) true cow-pox was

so much more widespread than at present that at the commencement of the century Jenner confidentially looked forward to its occurrence every year, and that in consequence the majority of milch-cows were liable to become infected at one or another period of their existence, this empirical method of Thiele's bears a curious and noteworthy resemblance to one of the methods proposed by Koch for the treatment of rinderpest. This consists in the injection into susceptible animals of a mixture composed of a small proportion of virulent blood diluted with a much larger quantity of serum obtained from an immune animal. Milk, of course, is merely an emulsion due to suspension of fatty particles in a fluid which may be regarded as blood serum; so that it is conceivable that milk obtained from a cow which had suffered not long before from cow-pox, and so become, for the time at any rate, immune, might so far modify smallpox lymph added to it, as to produce some such effect as noted by Thiele when the mixture was used for purposes of inoculation. I have been unable to find any records dealing with a repetition of these experiments, but it would be interesting to make investigation of the possibility of producing a similar lessening in virulence of smallpox lymph by admixture with the blood-serum of a calf which had been vaccinated some few weeks beforehand.

Ceely, in February 1839, inoculated three stirks with variolous lymph, with the result that in two of the three he eventually obtained, in due course, what he considered to be vaccine vesicles. From these, lymph

stocks were established, which at once came into such extensive use that in the course of a few months at least a couple of thousand children had been vaccinated therefrom. In his paper recording these experiments, he states that on former occasions, even though working purposely at different times of the year, he had altogether failed in attempts to variolate the cow. In order to test the measure of protection afforded to these children by his variola-descended lymph, Ceely tried on a number of them the effect of subsequent variolous inoculation at varying intervals of time from their vaccination. In no case did he obtain any greater result locally than, on the authority of Willan, is known to have attended the test-inoculations employed by Jenner and Woodville.

Unfortunately, owing to the particular methods used by Ceely in his variolous inoculations of cows, some doubt is thrown on the value of the results obtained by him. He was, according to his own account, working simultaneously with both variolous and vaccine lymph, so that it is difficult to exclude the possibility that his instruments may have unintentionally served to convey the infection of both diseases at one and the same time.

Several years before the commencement of his inoculation experiments, Ceely had made trial of a method originally employed by Sonderland of Barmen (1830), who claimed to have infected cows with the contagion of variola, by way, presumably, of the respiratory tract. This he did by enveloping them in blankets taken from the bed in which a patient had died of smallpox, and by hanging other blankets

similarly infected around the head of each cow, in order that the animal might breathe in the effluvium arising therefrom. It is stated that these cows subsequently manifested symptoms of cow-pox, and that from this source lymph was obtained and used on the human subject with the result that typical vaccine vesicles were produced. Ceely failed, however, in his attempt to bring about infection in similar fashion, as, indeed, have numerous observers in various parts of the world. Yet Ceely has himself recorded an occurrence observed by him in 1840, which although apparently but little known is of interest in this connection. He relates an instance in which five out of eight milch cows sickened with cow-pox within twelve and fourteen days of their having been seen to be licking over a quantity of flock from the mattress on which a patient had died of confluent smallpox, and which had been spread out in the field for purification. Careful inquiry at the time appears to leave no doubt that the animals, which had been on the same farm for a considerable period, were in good health at the time of their admission to the meadow in which the bedding was exposed, and also that there had not been any other case of cow-pox in the neighbourhood. The fact that all the animals became affected simultaneously, and after a period corresponding to the usual incubation period of variola, certainly affords some reason for the assumption that the outbreak owed its origin to the animals being exposed to the "effluvium" from the infected bedding. It is of course possible that infection by way of the digestive tract may have been

a factor also in this case, since Chauveau and other observers have shown experimentally that such result may be possible, a number of cases having now been recorded in which vaccinia has been communicated both to the human subject and also to the lower animals by the ingestion of vaccine lymph or powdered vaccine crusts.

There surely has seldom been so indefatigable a repeater of experiments as Badcock the Brighton chemist, who during a period of about twenty-five years performed more than five hundred variolous inoculations. In December 1840, Badcock, who had commenced his work without knowledge of Ceely's slightly earlier work, first succeeded in variolating a cow, from which he carried on a stock of what was indistinguishable from genuine vaccine lymph. Although he subsequently met with similar success on no less than thirty-seven separate occasions, the difficulty and uncertainty of the work will be appreciated on consideration of the fact that those experiments, which eventuated in success, represent but 7 per cent of the total number. The lymph obtained by Badcock as the result of his variolation experiments has been largely employed; he supplied it to hundreds of medical practitioners, and many thousands of children are said to have been successfully vaccinated with it. About twelve years (1852) after Badcock had obtained his first successful result, two American physi cians, Drs. Adams and Putnam, were equally fortunate, and were able in consequence, as reported at the time in a Boston daily paper, to furnish all the vaccine matter requisite for use in the city and neighbourhood.

In 1865 a Commission, appointed by the Society of Medical Sciences at Lyons, having Chauveau as its head, reported the results of a comprehensive series of experiments carried out by it during the two preceding years, the results of which were completely at variance with those obtained by previous investigators. These results have been admirably summarised in the Report of the English Royal Commission on Vaccination. "Inoculation of the cow with smallpox matter in any one of the thirty animals used did not give rise to a vaccine vesicle; nevertheless, a definite result was obtained in the form, however, not of a vesicle, but of a thickening and inflammation of the wound; when a puncture had been made this became a papule; lymph squeezed from such a papule, and inserted into a second animal, gave rise to a like papule; and this again might be used for a third animal, but often failed; and the effect could in no case be carried on through more than three or four removes. When the inoculation was repeated on an animal on which a previous inoculation had produced such a papule, no distinct papule was formed; and, moreover, lymph squeezed from the seat of the latter inoculation produced no effect at all when used for the subsequent inoculation of another animal." There is evidence that the development of the papule was the result of the specific action of the virus. This inference is strengthened by the fact that no such papule was produced by the Lyons Commission when the smallpox matter was inserted into an animal which had previously had cow-pox naturally or artificially; as well

as by the fact that when an attempt was made to vaccinate, with vaccine lymph of proved efficacy, an animal on which a papule had been so developed by inoculation with smallpox matter, the vaccination failed, though the animal had never had natural cow-pox nor been vaccinated. The specific nature of the lymph of the "Lyons" papule is held to be shown by the fact that such lymph, when used on the human subject, gave rise to smallpox. On the other hand, it has been urged that in this case the virus producing the effect was simply the original smallpox matter used in the inoculation, producing the papule and still clinging to the wound. This, however, is considered to be disproved by the experience that lymph from a "Lyons" papule of the second remove also gave rise in the human subject to smallpox. Thus Chauveau and his Commission found that smallpox implanted in the bovine animal gave rise to a specific effect which was not cow-pox, but was of the nature of smallpox; though its manifestations in the cow were different from those of smallpox in man.

A few years earlier, in 1860, Martin of Boston, U.S.A., appears to have had a somewhat similar experience, as about fifty persons vaccinated by him from vesicles raised on a cow's udder by inoculation of variolous matter taken from a pock on the body of a man who had died of the disorder, were nearly all attacked with smallpox, and three died.

Voigt, the Director of the Hamburg Vaccine Establishment, in 1881, brought into extensive use not

only in Hamburg, but also in other parts of Germany, a strain of lymph of variolous origin. In the early summer of that year he inoculated three calves with lymph obtained from a case of smallpox. In two of these the operation was apparently unsuccessful, in view of which it is of interest to note the fact that all three calves were afterwards found to be insusceptible to vaccination. In the third calf, a single vesicle appeared on the site of one, out of five incisions made on the left side of the perineum. Voigt adds, however, that this same animal had been vaccinated in one small place in the right hypochondrium (for what reason is not obvious), and that at this point a normal vesicle developed. Voigt does not consider that this fact invalidates the apparent success of his experiment, for the reason that, as he says, the lymph derived from, as he considers, the variolous source proved to be more potent, as regards its observed effects on children and calves, than the Beaugency vaccine lymph, which he had in current use at the time his experiment was carried out. Both Chauveau and Berthel have, on the other hand, expressed the opinion that Voigt's variolo-vaccine is merely a continuation of the strain of animal vaccine, previously employed in Hamburg.

In 1885 Simpson succeeded in an attempt to inoculate a cow with smallpox lymph obtained from a young vaccinated girl, but, so far as I am aware, he did not publish the details of this experiment at the time. Dr. Cory, to whom Simpson sent points charged from the cow on the sixth day after inoculation, has recorded the

results following on their use in his recent volume on *Vaccination.* Three days after receiving the points, Dr. Cory employed them at the Government Animal Vaccine Establishment, Lamb's Conduit Street, for the vaccination of a young male calf. Three out of five insertions made with the lymph resulted in vesicles indistinguishable from those produced by the current lymph of the Establishment. A second calf and also a child were therefore vaccinated from the first calf, with successful results in each instance, although in the calf only six vesicles resulted from seventeen insertions of the lymph. From this calf a continuous series of calf vaccinations were carried on during a period of six months. With the lymph obtained from these animals, 79 in number, no less than 1247 children were vaccinated, 1174 of whom afforded an insertion success-rate of 98.4 per cent.

Some years later (in 1892) Simpson, then resident in Calcutta, was again successful in raising a stock of vaccine lymph of variolous origin. Lymph was taken from a smallpox patient on the fifth, sixth, seventh, and eighth days of eruption, and each day a young calf was inoculated, the animal having been taken into the smallpox ward for that purpose.

The inoculations with the three later lymphs were unsuccessful, but the first calf showed on the sixth day of inoculation three papules on the abdomen and in the groin, at points where no punctures or incisions had been made. These on the following day had developed into vesicles. Two of the incisions and one puncture also contained lymph. On the sixth day lymph was

taken (whether from vesicles, incisions, or puncture, or all three is not clear from Dr. Simpson's paper) for the inoculation of another calf, from which again, on the sixth day, a child was vaccinated, the two insertions giving rise in due course to vesicles. From this child the strain of lymph was carried on successfully through nine generations, and Dr. Simpson remarks that each child showed splendid vaccine vesicles, much more typical in every respect than those obtained from the lymph, which had been previously in use.

In 1886 and 1890, Fischer, the Director of the Institute for Vaccination at Carlsruhe, obtained two strains of variolo-vaccine, as the result of inoculation of calves with human smallpox lymph. In the first series, Fischer inoculated a young female calf, which he took into the smallpox ward of the Pforzheim Hospital for this purpose. Typical vaccine vesicles had developed on the sixth day, the lymph from which was carried on through twelve generations in the calf, after which it was successfully employed for the vaccination of children. Fischer states that the initial experiment of his second series was carried out in the Carlsruhe Institute, but that the animal was placed in a separate stable, and that every precaution was adopted to secure freedom from vaccine contaminations. In this calf, directly inoculated with smallpox lymph, he again obtained, as in his experiments of four years previously, a vesicular eruption, having the characteristic appearance of ordinary vaccinia.

With lymph taken at the third remove from this

calf, he vaccinated his own grandson with complete success, and the strain, which has now been continued through a very large number of animals, has been brought by Fischer into general use.

Surgeon-Major King in 1889, being then stationed at Madras, inoculated a young bull-calf with fifth day smallpox lymph obtained from a child suffering from the disease. On the eighth day after the inoculation, a crop of vesicles made their appearance, not at points of insertion, but some little distance away, from the lymph obtained from which another calf was then inoculated. In this calf again secondary vesicles appeared, but, in addition, vesicles also formed at each point of insertion of the lymph. The lymph stock was carried on through three more generations in calves, the vesicular eruption being now in each instance strictly localised to the points of insertion. With lymph obtained from the fifth calf of the series, a number of children were vaccinated successfully. Dr. Simpson reports that from the stock thus established by King, 4240 British and native soldiers have been vaccinated, and also a very large number of the native civil population, with most gratifying results; the percentages of success being much above that obtained from the lymph previously in use. For reasons which are unknown to me, King's investigation appears to have been regarded with disfavour by the Government of Madras, by whom he was ordered back to his regiment and deprived of his special emoluments. Subsequently, however, this injustice was repaired, by the reinstatement of Surgeon-

Major King in his former position as Director of Vaccination in the Presidency.

In May 1892, Hime published the results obtained by him in a single experiment on variolation. The lymph employed for inoculation of the first calf was collected from a semi-confluent case of smallpox in a previously vaccinated woman of thirty-seven years old. No details are given as to *where* the experiment was conducted; but presumably it was the private establishment from which Dr. Hime supplies vaccine lymph, as he makes the statement that "the calf was placed on the ordinary vaccination table used for these animals." A number of skin incisions were made, into which the variolous lymph was inserted. Of these incisions all subsequently healed, being on the sixth day only "detectable to the touch by a slight roughness." Several "pocks," however, developed at points for the most part distinct from any of the incisions, and material taken from these on the ninth day of inoculation was employed for the direct inoculation of another calf. All the incisions made on this second calf had "taken" on the fifth day, when lymph was collected in tubes, and pulp removed by scraping. Some of the latter made into "conserve" with glycerine was sent to Fischer of Carlsruhe, who obtained excellent results with it on a calf, from which children were in turn successfully vaccinated. Hime also himself vaccinated a child with a capillary tube of the lymph in two insertions, at each of which points a typical vesicle presented itself on the eighth day.

The largest amount of research work in this direction during recent years is that of Haccius and Eternod (1893), of whom the former is the Director of the Swiss Vaccinal Institute at Lancy, Geneva, where the work was carried out. Haccius gives an account of no less than seven successful series of experiments, in each of which a strain of variolous lymph, obtained in the first instance from the human being, was carried on from calf to calf through, in some instances, as many as six or seven removes. The general result was that by the second, or at the most the third, remove, the effect produced was altogether indistinguishable from that which we are accustomed to see in a calf that has been successfully vaccinated. With the lymph of the fifth or sixth remove, a number of previously unprotected children were vaccinated with unfailing success. The resulting vesicles could in no way be distinguished from ordinary vaccination vesicles of equal age. The total number of animals employed in these experiments is not mentioned, but from certain statements in M. Haccius' publication, one gathers that it was very considerable. It is obvious, therefore, that the Lancy experience, as to the small proportion of success to failures, is similar to that of other observers. It is worthy of note, also, that in those animals in which inoculation of variolous matter appears to have been attended with success, no definite vesiculation was ever observed at the site of any of the punctures or incisions that were made. The usual result in the first remove from the human being was either a single vesicle or

group of vesicular points on the site of a scarification or denudation of the skin. It is particularly stated that every precaution was taken, as, for instance, by the sterilisation of the lancet before each operation, to prevent the accidental transmission of vaccinia.

Klein, who in 1879 had obtained in thirty-one trials what then appeared mere negative results, renewed his investigations in 1892, employing now calves for inoculation experiments instead of milch cows, as in his earlier attempts. Having procured at the Hospital Ships, off Dartford, lymph from the vesicles of two cases of confluent smallpox, one at the sixth, the other at the seventh day after appearance of the rash, this lymph was, on the same day, inoculated by linear cutaneous incisions on two calves at the Brown Institution. The inoculation of one of these calves afforded no indication of success, but on the other calf he obtained a local result in the groin, which, however, consisted not of a distinct vesicle, but merely a thickening and redness, together with a linear crust, at the site of certain of the incisions into which smallpox lymph had been introduced. Lymph pressed from the thickened wounds, when inoculated into a second calf, produced also by the fifth day a like but rather more marked result; while the thickening and redness still further increased as the process was repeated in a third and fourth calf. In none of these animals was there any appearance of vesiculation. At this stage the fourth calf of the series was removed to Lamb's Conduit Street, where Dr.

Cory, with material obtained by clamping and scraping the thickened incisions on the seventh day, vaccinated an infant in five separate insertions, with complete success. Each of these presented on the eighth day appearances characteristic of vaccinia. Moreover, Dr. Cory, who kept the child under observation from first to last, was unable to detect in the course of the disorder any deviation from the condition of affairs normal to vaccinia. "Crusts" from the arm of this child were preserved and utilised, after being mashed up in sterile salt solution for the retro-vaccination of another calf. In due course there resulted, in about half of the total number of incisions, vesiculation of a character not to be distinguished from that current in the calves vaccinated in the ordinary course at the Lamb's Conduit Street Station. Finally, after a lapse of six weeks, Klein submitted this retro-vaccinated calf and other calves vaccinated from it to the test of a further and thorough vaccination with current calf lymph, and with the result that all these animals entirely resisted vaccinia. I have learned privately from Dr. Klein that, in his former (1879) experiments on cows, he in more than one instance obtained results somewhat similar to those witnessed in the calf on which his more recent successful inoculation was performed. He suggested at the time the advisability of attempting a transference to a second animal, but Ceely, who was superintending the experiments, insisted on the necessity of obtaining a vesicle in the first animal inoculated as the only criterion of success, and the suggestion was therefore

overruled. For his own satisfaction, however, Klein endeavoured to vaccinate one of the cows which had shown some local reaction, but entirely without result, the animal apparently being immune.

Of my own experiments the most successful series may briefly be described as follows:—With variolous lymph (contained in two capillary tubes) taken on 30th July 1892 from a vaccinated girl, aged sixteen years, at from the fifth to the sixth day of the eruption of discrete smallpox, I inoculated on 11th August a cow-calf A, about six weeks old, by thirty-two linear incisions and two superficial scarified patches made on the abdomen. On 13th August (the third day) this calf presented nothing but a slight scab over the scarified patch. On 15th August (the fifth day) some of the incisions were somewhat red and elevated, more particularly at several definite points which tended to be vesicular. At this date, on the inner aspect of each thigh, and distant in each instance from incisions or scarifications, was a crop of shotty and incipiently vesicular pimples. On 17th August (the seventh day), having clamped three of the incisions, including those with the quasi-vesicular appearance, I removed the lymph with the crusts by scraping, and used the material for the inoculation on this date of calf B. The accessory incipient vesicles I left untouched. From first to last calf A never exhibited any appreciable rise of temperature or indisposition of any sort. On the ninth day the linear incisions, the cross scarifications, and the accessory vesicles of calf A were in their decadence, and in a few days more the

whole process was at an end. More than a month later, namely, on 22nd September, I vaccinated this calf A in twenty-three linear incisions, with calf lymph obtained from the Animal Vaccine Establishment. The result was absolutely negative.

On 17th August, with the scrapings obtained the same day from calf A, I inoculated a cow-calf B, two months old, in fifty-six linear incisions on the abdomen. On the third day every one of these incisions was distinctly raised and bordered by a delicate pink flush. On the fourth day (20th August) all incisions had evidently "taken," the areola about each being now well marked, and about one-eighth of an inch in width, but there was no definite vesiculation. At this date, after clamping, scrapings were taken from certain of the incisions for further experiment. No accessory vesicles appeared in this calf, which, along with calf A, was vaccinated on 22nd September in seventeen linear incisions with calf lymph obtained from the Animal Vaccine Establishment. The result, as in calf A, was absolutely negative.

On 20th August, with scrapings taken the same day from calf B, I inoculated a small cow-calf C (7 weeks old) in twenty-seven linear incisions on the abdomen. On the fourth day (23rd August) every incision had "taken," and in most of them there was evidence of commencing vesiculation. Two days later (25th August) vesiculation was distinct in several of the incisions. At no time were there any accessory vesicles. This calf also was vaccinated (22nd September) with calf lymph

from the Animal Vaccine Establishment. Again the result was altogether negative.

I have thus far obtained an undoubtedly successful result in one series only out of four attempts, but I have at any rate been able to satisfy myself that it is possible to variolate the calf, and, further, that the result obtained in the first instance may become greatly modified in the course of successive removes; and, again, that animals which have been thus treated are no longer susceptible to vaccination.

I may perhaps mention here that all my experiments were conducted at the Brown Institution, to avoid any possibility of contamination with vaccinia; that, as a further precaution, new scalpels were used, which were invariably first carefully sterilised in a flame; that before and after use the table was thoroughly washed with carbolic acid and hot water, and during the intervals of use kept exposed to the open air.

The Royal Commission on Vaccination, in the section of their report dealing with the question of the relationship of variola and vaccinia, show that the various series of inoculation experiments, of which I have given a brief account, fall into one or other of three categories. The first category includes the experiments of Thiele, Ceely, Badcock, Voigt, Haccius and Eternod, King, Simpson, Hime, and others. In these experiments "inoculation of smallpox matter into the udder, or adjoining parts, of the bovine animal gave rise, at or near the site of inoculation, to a vesicle, either identical in visible characters with the ordinary vaccine vesicle produced

by inoculation with the matter of cow-pox, or to a vesicle the features of which, while not corresponding wholly with those of a perfect vaccine vesicle, so closely resembled it as to justify the recognition of the vesicle as a vaccine vesicle. Also it includes experiments in which, though the local result had not the characters of a perfect vaccine vesicle, yet lymph from it, when carried through a second or third remove in the cow or calf, presented results fully manifesting those characters, and when again transferred to man gave results indistinguishable from the ordinary vaccine vesicles." In the second category are placed the experiments of Klein and myself. In no instance did either of us obtain any appearance of vesiculation in the animals directly inoculated with smallpox virus; although the appearance of the eruption tended to resemble more and more that typical of vaccinia, the further the remove, through a series of calves, from the original strain. In the third category are included the practically negative results of the Lyons Commission, obtained by Chauveau and his fellow workers. A brief account of these has already been given.

With the exception then of Martin and of Chauveau and his colleagues of the Lyons Commission, all the observers mentioned claim to have obtained positive results, in a certain number of their experiments, at any rate, as regards the production of typical vaccinia, after one or more removes, as the result of variolation of the cow or calf. By no one, apparently, has success been attained invariably; but it is among the experiments of

the earlier observers especially, who made use, for the most part, of heifers and milch cows, that the largest proportion of abortive attempts are to be met with. Subsequent experience has shown that success is much more likely to be attained if calves be used instead of heifers or cows. In this way, perhaps, Chaveau's somewhat anomalous results may be in part explained.

With reference to recent experiments on variolation of the calf, it is worthy of note that, as previously mentioned, different observers have obtained local effects in that animal which, in different calves of a series, have varied considerably. The final result has, however, after a greater or less number of removes from calf to calf, been invariably the same; namely, a local vesicle is produced which by no means at our command, such as the appearance and course thereof, or the protective power of the lymph derived therefrom, is distinguishable from true vaccinia.

Although practically there is unanimity of opinion among those who have worked at this subject, it must be confessed that, seeing the conditions under which they were carried out, many, particularly of the earlier experiments, are of little worth. Some of the main objections are based on the fact that experiments on this subject have almost without exception been performed in establishments devoted to the continuous cultivation of vaccinia; on the use concomitantly of vaccine and of variolous lymph on the same animal; and on the want of care as to the cleanliness and freedom from vaccine contamination of lancets and

"points" used in the experiments. Objection of similar sort against the variolations of the calf, which have been achieved in recent years, is hardly valid. In some of the more recent cases, at least, special precautions have been taken to ensure that the instruments and table were sterilised, and to render the environment of the animal such as to afford no likelihood of the communication of vaccinia. Most especially was this so as regards Klein's investigations and my own. The number of successful cases which have been recorded is now so large that it is difficult to believe that sources of fallacy of the above sort should have been present in every instance; and it is therefore well-nigh impossible to resist the conclusion that a change of smallpox into vaccinia must really have come about.

Nevertheless, there are found even at the present day those who, like Juhel and Dupuy (1894) and Layet (1895), maintain the essential duality of variola and vaccinia. It is, however, difficult to understand how the results obtained, by the last-mentioned especially, can bear the interpretation he would place upon them, seeing that he obtained, as the result of inoculating heifers with smallpox lymph, a vesicular eruption, the lymph derived from which reproduced the disease on other heifers. Moreover, subsequent vaccination of all his series of animals was either abortive or wholly without result.

Hervieux (1895), the Director of the Vaccine Institute attached to the Académie de Médecine at Paris, takes up a similar position to that of Layet and his collabo-

rators, Le Dantec and Benech. He asserts that the only point of similarity between variola and vaccinia, which has been brought forward by those who believe in the relationship of these disorders, is the likeness between the vesicles which are found in either case, and denies that this point forms any argument in favour of their identity. He also considers that the use of lymph of variolous origin is fraught with danger, and so, doubtless, it may be when that employed is derived, as in Chauveau's and Martin's cases, for instance, directly from the animal first inoculated.

If, as I believe, it can be conclusively proved that smallpox lymph, by passing through the system of the calf, can be so altered in character as to become deprived of its power of causing a generalised eruption, while inducing at the site of inoculation a vesicle indistinguishable from a typical vaccine vesicle; and, more important still, if it be shown that when transferred again to man, it has by such treatment completely lost its former power to produce a general disease, it may fairly be asserted that cow-pox—or rather that artificially inoculated form of the disease which we term vaccinia—is nothing more nor less than variola modified by transmission through the bovine animal. Perhaps the most reasonable interpretation of such results may be that smallpox and vaccinia are both of them descended from a common stock—from an ancestor, for instance, which resembled vaccinia far more than it resembled smallpox. It is conceivable, indeed, that the seeming vaccinia, obtained in the calf by inoculation of small-

pox matter into that animal, may after all be but a reversion to an antecedent type; and in this connection we may call to mind a fact of universal experience, namely, that vaccinia, however it may have arisen in the past, or is made to appear in the present, exhibits little tendency to "sport" (as, for instance, by manifesting a "generalised eruption") in the direction of small-pox.

Mr. Picton and Dr. Collins, in their addendum to the Report of the Royal Commission on Vaccination, lay much stress on the want of "evidence to show that inoculation of the pox of the cow on the human skin has ever produced smallpox." Variola and vaccinia may, nevertheless, have a common ancestry, since it is not unlikely that variola may have departed widely from the original type, and have gained an exalted virulence by repeated passage through man under conditions favourable to its propagation and activity. If this evolution of the disease has, in fact, taken place, variola may have suddenly reverted, under greatly changed conditions (as for instance implantation on the bovine animal), to an ancestral type. But the reverse process (*i.e.* sudden "sport" of vaccinia in the direction of smallpox) is not to be expected. It is most unlikely that a less differentiated form (cow-pox), also emanating from the common ancestral stock, should attain to the most exalted virulence in a single individual, and *per saltum* declare itself as smallpox, as the dissentient Commissioners insist that it ought to do.

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CHAPTER III

HISTORY OF VARIOUS LYMPH STOCKS

JENNER'S first case of vaccination was, as previously stated, that of a boy eight years of age, whom he inoculated in the arm with cow-pox matter taken from the sore on the hand of a dairymaid, who, in turn, had become infected with the disease from milking cows suffering from cow-pox.

This was in 1796, and was his first actual experiment purposely performed, though it is evident that for years previously he had taken note of the results of the testing of many experiments unintentionally performed by milkers on their own persons, such experiments being subsequently tested at a longer or shorter interval by inoculation with smallpox, the inoculation being itself in many cases performed not purposely as a test, but as a preventive of smallpox in the course of the ordinary practice of some medical man in the dairy districts. In experiments and tests so conducted, there could be no "personal equation" to confuse the result.

It was apparently not until 1798 that he made his first attempt to carry on a strain of lymph from arm to arm. In the spring of this year he inoculated a child

with matter taken directly from the nipple of a cow; and from the resulting vesicle on the arm of this child first operated on, he inoculated, or, as it may now be more correctly termed, "vaccinated" another. From this child several others were vaccinated; from one of these a fourth remove was carried out successfully, and finally a fifth. Four of the children were subsequently inoculated with smallpox—the "variolous test"—without result.

At this point, however, the strain appears to have been allowed to die out; but, in January 1799, Woodville, physician to the Smallpox Hospital in London, who had been much interested in Jenner's investigations, discovered the presence of cow-pox in a dairy in Gray's Inn Lane. With lymph taken, in the presence of Sir Joseph Banks, the President of the Royal Society, and other well-known men of the time, from one of the cows in this dairy, he vaccinated seven persons at the Smallpox Hospital; while in the case of certain other persons, he employed matter from sores on the hand of a dairymaid, who had become infected from one of the cows at this same place. These cases, from which afterwards in succession many hundreds of persons were vaccinated, were the main source of what is usually spoken of as "Woodville's lymph."

These strains of lymph were extensively distributed by Woodville. Dr. Pearson, one of the surgeons to St. George's Hospital, also sent out much lymph.

He very early obtained lymph from a dairy in the Marylebone Road. It is not unlikely also that a con-

siderable part of his lymph was obtained from Woodville's cases. Jenner himself used some of Woodville's lymph, and he obtained a further supply from a cow at Mr. Clarke's farm in Kentish Town.

The lymph first employed on the Continent and in other foreign countries was undoubtedly supplied in large measure by Pearson and Woodville; although we learn from Baron and other authors that Jenner, who was naturally much appealed to for supplies of lymph, himself sent lymph to Stromeyer of Hanover, to De Carro of Vienna, to Berlin, and to Barbados, Newfoundland, and other parts of America.

The lymph which Woodville first sent to Paris died out, and he himself visited France with a fresh stock, taken at a time when he had learned to be careful to avoid contamination of his stock by variolous matter.

Strains derived from lymph stocks originally supplied by Jenner were also sent abroad by a number of different persons, the original strain being, in large measure at any rate, the lymph obtained by Jenner from the Clarke's farm cow. Thus Ring records that he distributed Jenner's lymph to various places on the continents of Europe and America.

It would be erroneous to suppose, however, that all the lymph employed abroad in the early days of vaccination was obtained from England. Indeed, both Sacco and De Carro made extensive use of lymph obtained by the former from a case of natural cow-pox which he discovered in Lombardy. In a letter

to Jenner, under date 16th October 1801, Sacco states that from this source "more than eight thousand inoculations" had already been "performed with the most happy success," and he adds "several hundreds of these have since been subjected to the variolous inoculation, and have resisted it." In a postscript he adds that he had sent some of the Lombardy vaccine matter to Woodville. From this stock also De Carro sent supplies to Constantinople, where it was employed for the first vaccinations carried out in this part of Europe. De Carro it was also who first succeeded in conveying a supply of lymph to India. This lymph, again, was not from Jenner's stock, but was of Milanese origin, having been furnished to him by Sacco. It was, moreover, not of bovine but of equine origin, and according to De Carro had never been passed through the cow.

This strain of equine lymph was originally obtained by Sacco in 1812, from a crop of vesicles on the hands of a coachman who had not had smallpox, and who had dressed the heels of a horse affected with the "grease." From these vesicles two children were successfully vaccinated, and from these the stock was carried on through a long series of vaccinations.

Among more recent strains may be mentioned that obtained in 1836 at Passy in the environs of Paris from the hand of a milker, who had contracted casual cow-pox.

The old stock then in use at the Académie de Médecine had evidently degenerated somewhat; and, when its effects were compared with those of the new

Passy lymph, the vesicles developed from the latter were found to be manifestly finer.

In 1836, Estlin of Bristol put in circulation a stock which at first showed unusual activity. This abated, however, after some transmissions, and the lymph afterwards came into extensive use.

From this time onwards the various stocks became so numerous that Ceely, writing in 1841, states that during the preceding three years he had experimented with lymph from more than fifteen distinct sources; of these six had been taken from the natural disease, either direct from cows or from vesicles on the hands of the milkers, and seven were artificially produced in the cow.

The lymph stock in use at the present time at the Government Animal Vaccine Establishment was originally obtained on 26th November 1881 at a farm in the village of Lafôret not far from Bordeaux; whence a sample of lymph from the seventeenth calf in succession from the animal first affected was sent by Dr. Dubreuilh of Bordeaux, to the Medical Officer of the Local Government Board.

Of late years, more particularly, numerous strains of so-called variola-vaccine lymph, obtained by inoculation of human smallpox on the calf, have been introduced, especially by Fischer in Germany, by Haccius in Switzerland, and by King in India. These strains have been successfully transmitted through many thousands of individuals.

Those who deny the relationship of smallpox and cow-pox will say that these children have been variolated,

and not vaccinated. One, of course, admits that they *have* been variolated, in the sense that they have been inoculated with lymph descended from a case of human smallpox, but differing from the mild inoculations of Adams, Dimsdale, and the Suttons, in that the resulting disease is no longer infectious. Such procedure is also strictly comparable with those methods of protective inoculation by the use of attenuated virus, which of late years have given valuable results in the prevention and treatment of various zymotic diseases.

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CHAPTER IV

HISTOLOGY OF THE VACCINE VESICLE

During the evolution of the local changes which result from the insertion of vaccine lymph beneath the surface of the skin, it is possible to recognise three more or less definite stages of papule, vesicle, and pustule.

The same statement holds good with reference to the eruption of smallpox, whether this be local, *i.e.* due to inoculation of the virus, or general, as the result of infection.

In each instance the appearance of the first or *papular* stage is brought about by inflammatory reaction, causing an increase of intercellular fluid together with concomitant increase in volume and number of epithelial cells of the rete Malpighii more particularly. The papule gradually becomes enlarged by a circumferential extension of the same process, and owing to further changes in the cells first affected. Vacuoles arise in the central portion of the papule, by the extension of which this ultimately becomes a vesicle.

The *vesicle* is a multilocular structure, the dissepiments, by means of which its interior is divided up, being

formed from the thinned and extended remains of the original epithelial cells. Owing to the fact that the process of vacuolation, for a time, increases more extensively at the advancing edge of the vesicle, the central portion remains somewhat less elevated, thus giving rise to the appearance termed umbilication.

Stanley Kent, as the result of histological examination of a series of vaccine vesicles, which I had removed from the calf at gradually increasing intervals of time after vaccination, showed that at a quite early stage of the process an outflow of leucocytes takes place, towards the point of injury. In time each blood-vessel becomes the centre of an aggregation of leucocytes, which by the rapid increase in their numbers eventually transform the originally clear inflammatory exudation into a purulent fluid. The vesicle is said now to have become converted into a *pustule.*

By the thinning and ultimate rupture of its trabeculæ, the pustule finally becomes unilocular. The turbid fluid contained in it now gradually dries up and, together with the necrosed remains of epidermal cells, takes part in the formation of the *crust*, which under the microscope appears as a homogeneous mass very deeply coloured by the ordinary stains.

Meanwhile a regeneration goes on underneath the crust, the new epidermis being formed by an ingrowth from the surrounding stratum lucidum. The extent to which the cutis vera has been involved determines the depth of the resulting scar.

The minute local changes produced in the skin of

the calf by vaccination has recently been made the subject of careful study by Dr. Gustav Mann, who also employed for the purposes of his research specimens of skin removed by me at varying intervals after the operation of vaccination. His description of the normal skin of the calf, and of the changes produced in it by vaccination, is based on the examination of material which was fixed, immediately after removal, in picric acid and corrosive sublimate mixture and cut in paraffin.

The abdominal skin of the calf exhibits a sharp demarcation into epidermis, dermis, and hypodermis.

The epidermis is composed to one-fifth of its thickness of the stratum corneum, then follows a single layer of cells with the characteristic granules of the stratum granulosum, and finally the stratum Malpighii, built up of cells three to four layers deep. Of these cells those nearest the dermis have long finger-like processes fitting into a very dense layer of white fibrous tissue. Weigert-Kromayer's staining method reveals fibrils which in the youngest layer of epithelial cells are arranged vertically, while in the second and subsequent strata they assume a horizontal course, giving rise to the "prickly" appearance and uniting the cells very firmly.

The dermis is composed (1) of the already mentioned dense outermost layer [basal membrane] to which the epithelial cells are attached; (2) of a very loosely arranged connective tissue, consisting of fine bundles of collagenous fibrils with clasping cells, which

latter lie with their wing-like processes parallel to the surface of the skin; and (3) of an inner elastic zone distinguished by the presence of coarse elastic fibres which for the most part run horizontally. These fibres give off fine twigs, which pass obliquely upwards into the papillary zone, and also form densely felted cylinders in the outer root sheath of the hairs.

The hypodermis is chiefly composed of thick bundles of collagenous tissue, with a few elastic elements in the septa separating the bundles one from another. In the external region of the hypoderm is found a plexus of very large lymphatics.

Vaccination, in all the specimens examined, has resulted in a complete division of the epidermis, the vaccine lymph employed being therefore brought directly into contact with the loose dermal tissue, and it will be seen that the chief changes are brought about in that zone which lies between the hypoderm and the basal membrane.

In a specimen removed within an hour after vaccination, the wound is blocked by a clot which externally is of a coarsely granular nature; between the edges of the epidermis it is finely granular, and in the dermis is found a close fibrin reticulum, denser at its periphery than in the centre, and enclosing numerous red and a few white corpuscles. The blood-vessels close to the injury are dilated, and many of them completely thrombosed with a mass of white corpuscles. That already some change has taken place in the vessel walls is evidenced by the fact that close to the site of injury

red corpuscles may be seen adhering to the lumen of the capillaries and arterioles, while the blood from the rest of the specimen has escaped at the time of removal of the skin.

The nuclei of both the epidermal and dermal cells in the neighbourhood of the lesion are swollen, and the basophil chromatin contained within them is increased to at least double its normal amount.

In the dermis an emigration of leucocytes into the loose connective tissue spaces is noticeable. It is possible to demonstrate at this very early period a considerable number of diplo-bacilli and single very short bacilli in that part of the clot which lies between the edges of the epidermis and also in the somewhat œdematous connective tissue clefts quite close to the lesion.

After twenty-four hours the epithelium close to the injury has increased two to threefold in thickness, and already a phenomenon, which gradually becomes more and more pronounced up to the seventy-second hour, is seen, namely, the formation of Guarnieri's supposed parasites. In methyl blue eosin-stained sections the bluish nuclear chromatin is increased, but even more so the nucleolar chromatin, which stains a bright red colour with the eosin. By a process, which may be studied under normal circumstances in the pancreas, a considerable portion of this nucleolar matter leaves the nucleus and is found lying free in the cytoplasm ; in some cells one only, in others several granules are extruded. These are at first very minute, but, later,

they increase in size and occasionally seem to fuse; in this way there arise more or less solid spheres lying alongside the nucleus, or even indenting it.

From the twenty-fourth to the forty-eighth hour an area varying in width from one to two millimetres has become affected. The dermis shows a gradually increasing œdema, accompanied by an emigration of leucocytes, which results in a tumefaction of the outer dermis, rendering its structure even looser than normal. The deeper portions of the dermis, so rich in elastic fibres, show as yet practically no changes, and the hypodermal cells, except for a somewhat greater affinity for basic dyes, are also unaltered. As the result of this œdematous condition, developed in the neighbourhood of the site of inoculation, the lymph is prevented from escaping downwards by the dense elastic layer of the dermis and the thick collagenous bundles of the hypoderm. Towards the periphery the lymph channels are blocked by leucocytes, and there remains only one path open for the lymph, namely, through the basal membrane into the intercellular channels between the epithelial cells. There results thus a widening of the normal lymph channels up to the stratum corneum, and if it were not for the very resistant horny layer and the dried-up clot at the site of the incision, the lymph would readily escape in this direction.

It may be noted that during the second day many leucocytes are seen between the epithelial cells, differing from those found normally in being usually more or less spherical, rather than angular and elongated.

The changes most characteristic of vaccination are found during the third, fourth, and fifth days (72 to 120 hours after vaccination).

At the end of three days one may, for descriptive purposes, distinguish several zones. Furthest away from the line of inoculation the only change noticeable is a dilation of the inter-epithelial lymph channels. Immediately within this region all epithelial cells, except those of the stratum corneum, are much swollen and contain granules giving the same reactions as those found in normal cells of the stratum granulosum. We find thus a premature ageing of the cells.

The dermis underneath these cells forms large bullæ, the walls of which consist of compressed connective tissue cells, collagenous fibres, and numerous leucocytes. No wandering cells are found in the bullae, but fairly numerous diplobacilli and single bacilli.

Still nearer the point of inoculation the epithelial cells contain markedly enlarged nuclei, some of which before division reach a diameter two to three times greater than normal. As a rule, the nucleus fragments, however, into six to twelve smaller nuclei, which, lying closely together, become flattened against one another on one or more sides. Concurrently with the formation of these multi-nucleated giant cells one meets also with greatly distended lymph vesicles in the epithelium, the walls of which are composed of the enormously stretched fibrillæ of degenerated epithelial cells. In the interior of the vesicles, in addition to the fibrin reticulum, are found various micro-organisms.

Internal to the zone just described the giant cells are replaced by cells having only a fifth to a quarter the size of the multi-nucleated cells and possessing only one or two nuclei. These smaller cells are spherical, while the larger ones are angular in outline. It seems probable that the smaller globular epithelial cells are derived from the giant cells.

In the centre of the vaccinated area no living epithelial cells are present, but only remains of the stratum corneum and of a dense dried-up blood-clot.

The changes described for the third day also hold good for the fifth, the only difference being that the central necrosed area has increased in size and that the zone of infection has spread out laterally. By the continued emigration of leucocytes the central zone necroses more and more, the connective tissue elements succumbing to the pressure exerted by the wandering cells. The only elements which do not undergo any change during this death of the dermal elements are the elastic fibrils, which up to the very end stain quite sharply with orcein.

While up to the end of the second day practically no changes could be made out in the hypoderm, there is found later on, and especially about the fifth day, a considerable swelling of the thick white fibrous bundles, called forth by the great activity of the fixed connective tissue cells. These latter stain more deeply with basic dyes than normal cells, and their numerous branching processes will stand decolourisation more strongly.

No reference has been made, up till now, to what I consider the most characteristic feature of vaccination,

viz., certain appearances which are constantly seen in tissues fixed by picro-corrosive sublimate solution, and also, but not so well, when alcohol is employed for fixation. On staining sections in Löffler's methylene blue (in which the normal amount of caustic potash is increased ten times) for twenty-four to forty-eight hours at 30° C., and then differentiating for ten to thirty minutes in 1 per cent acetic acid, one finds immediately outside the necrosed area, in the superficial loose dermal tissue, a number of globular masses, varying considerably in size, and arranged either singly or in pairs.

The further outwards the smaller these globules become, till at the spreading zone one finds in the connective tissue clefts very short bacilli arranged singly or in pairs.

The idea that the larger globules represented either a capsulated, sporulated, or involuted stage of the bacillus led to the endeavour to demonstrate if possible a central corpuscle. After many failures the following method has been found efficient for this purpose. Sections must be stained in Unna's polychrome methylene blue for twenty minutes, then be differentiated by Unna's method in 30 per cent tannic acid for one to three minutes, according to the thickness of the section.

Although the globules vary in diameter, the stainable spot in the interior, which appears spherical or slightly elongated, is usually of the same size as the micro-organisms in the peripheral advancing area.

The exact nature of these bodies is being made the subject of further investigation.

Chemistry and Morphology of Vaccine Lymph

Fresh vaccine lymph, taken before full maturity of the vesicle, is a clear, transparent, limpid fluid, almost colourless in man, and slightly straw-coloured when obtained from the calf; this difference in colour depends on the varying quality of the normal pigment present in the blood plasma. Calf vaccine lymph is also somewhat more viscid than human lymph, and does not flow so readily when the vesicle is punctured, for which reason it is necessary to use compression forceps in the ordinary methods of collection of calf lymph.

Examined microscopically, vaccine lymph contains a certain amount of epithelial débris, a few cells and portions of cells being always visible. Leucocytes are usually present also, the number depending on the stage at which the lymph is taken; few or even none are to be found in the contents of the vesicle when first formed, but at or after the period of maturation they may be so numerous as to render the lymph turbid, or even puriform.

A few red blood corpuscles may be noticeable, although most observers will hardly agree with the statement made by Dr. Husband to the Royal Commission on Vaccination that this is invariably the case.

In stained specimens particularly, micro-organisms of one or more varieties can be readily demonstrated, the actual numbers being dependent to a certain extent on the care with which the lymph has been collected.

The nature and significance of the various microbes, which at one time or another have been isolated from specimens of vaccine lymph, will be discussed in the section on bacteriology.

It is matter of common knowledge that untreated lymph, when stored in capillary tubes, tends after a longer or shorter interval to become cloudy, under which circumstances it is also liable to be uncertain in its action if subsequently used for the operation of vaccination.

This opaque appearance may be quite independent of any coagulation of the lymph, as may not unfrequently be demonstrated on breaking tubes in which it is most marked. On the other hand, where clotting has taken place after the lymph has been stored, the opacity tends to form with the coagulum a central whitish thread in the midst of a clear fluid, instead of being distributed through the lymph in discrete points, as may otherwise be the case.

If cultivation experiments be carried out by inoculation on nutrient media, the number of colonies resulting from such inoculation with the contents of tubes which have become opaque is usually much greater than if fresh lymph is employed in a similar manner. We are apparently justified, therefore, in considering that the opacity of such old stored lymph is, in the main, the outcome of an enormous multiplication of aërobic bacteria, the ancestors of which are present in the lymph when first collected, although their numbers are then so comparatively small as not to render it in any

way turbid. It follows that ordinary vaccine lymph, which has become opaque, should never be employed for vaccination.

Vaccination lymph, chemically speaking, consists of the serum, or rather of the plasma of the blood. When freshly obtained, therefore, it is faintly alkaline in reaction, but it tends to become distinctly acid after a time when stored. In addition to the various salts and proteids normally present in the blood plasma, vaccine lymph contains some substance, possibly of the nature of a toxin or ptomaine, which results from the vital activity of the specific organism peculiar to vaccinia. That this is so may be proved either by filtration of the lymph through porcelain, or by exposure of it to a temperature of about 50° C. If, as I have shown, such filtered or sterilised lymph be inoculated on the skin in the usual manner, no obvious effect is produced at the point of inoculation, but it not unfrequently happens that the animal has been rendered temporarily immune to the effects of subsequent vaccination with lymph of normal potency. (See also Smallpox Antitoxin, p. 133.)

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CHAPTER V

BACTERIOLOGY

ALTHOUGH the study of the bacteriology of vaccinia and variola has, during the past thirty years or so, attracted the attention of a great number of observers, much yet requires elucidation before we can look upon our knowledge in this direction as satisfactory. Nevertheless progress, though slow, is perhaps sure, and it would seem as if it must be the very difficulties of the subject which enhance the inducement for the constantly renewed initiation of investigations by observers in all parts of the world.

As long ago as the year 1809, Sacco placed on record the fact that in vaccine lymph there might be found certain granules generally collected together into masses, and possessed of the power of automatic movement. The observation appears to have stood alone for more than half a century, but in 1863 Dr. Lionel Beale gave an impetus to the investigation of the morphological constituents of vaccine lymph by his description of the existence in it of transparent or hyaline particles of extreme minuteness. Both in his original paper in the *Microscopical Journal*

for April 1864, and also subsequently in the Report of the Royal Commission on Cattle Plague, Dr. Beale expressed the opinion that these granules constituted the contagious principle of the disease, but he does not appear to have obtained experimentally any facts in support of his belief.

A few years later Hallier and Zurn (1867) and Keber (1868) called attention to the presence in vaccine lymph of numerous micrococci, or "grains" as they were termed by the latter observer, who also evidently regarded the bodies found by him as the carriers, if not the actual generators, of the virulent principle.

It is, however, to M. Chauveau (1868) and Professor Burdon-Sanderson that we owe the first real step toward the elucidation of the micro-pathology of vaccinia, these observers having demonstrated that vaccine lymph, when freed from its contained particles, and inoculated on a living animal, no longer causes vaccinia; while, on the other hand, the precipitate or deposit, separated from it in one way or another, when employed in similar fashion, remains capable of producing the disease.

Chauveau found that vaccine lymph, taken at the stage of maturation of the vesicles, contained minute granular bodies, to which he gave the name of *corpuscles noirs*, and not unfrequently also much larger bodies resembling the leucocytes of pus. He further found that if the lymph was diluted ten times, and allowed to stand, these leucocytes, when present, gradually subsided to the bottom of the containing vessel, and

could then be removed. When this was done, and the material employed for an experimental vaccination, a local result was still obtained, thus showing that the active principle was contained not in these leucocytes, but either in the granules or in the fluid portion of the lymph.

In order to settle this point, Chauveau devised a method of diffusion by which he was eventually able to separate the granules from the fluid portion of the lymph. The amount of vaccine lymph at his disposal being necessarily exceedingly small, the ordinary means for obtaining diffusion were not applicable. Chauveau attained the desired object, however, by carefully floating a layer of distilled water on to the surface of a drop of lymph contained in a tiny glass vessel of cylindrical shape. If this manipulation is dexterously performed, the two liquids do not mix with each other excepting in the immediate neighbourhood of the point of junction of the two layers. But after a time the soluble constituents of the vaccine lymph pass upwards into the water, so that if, after twenty-four hours or so, a portion of the upper layer be carefully removed by means of a capillary tube, and examined under the microscope, it is found to be free from "granules." And further, Chauveau was enabled to demonstrate the fact that while this upper layer of fluid, which could be shown to contain albumen, and so afforded evidence of diffusion having taken place, gave negative results only on inoculation, the lower layer, on the contrary, which contained the "granules" in

suspension, was found to be active as vaccine, normal vesicles being obtained as the result of its inoculation.

Professor Burdon-Sanderson shortly afterwards repeated Chauveau's work, employing certain improvements in the method which seemed likely to render it more accurate, and thus to afford results of greater scientific value. He also made use of another method of his own, consisting in the filtration under pressure of the liquid to be tested through a layer of unglazed porcelain, by which means all solid particles were effectually separated from the fluid containing them. Professor Burdon-Sanderson, by the employment of this method, and also of his modification of that originally introduced by Chauveau, fully confirmed the results announced by the French investigator.

Further, Chauveau, by largely diluting vaccine lymph with distilled water, and employing the mixture for vaccination, found that he thus obtained strong additional evidence in support of the doctrine, now universally accepted, that contagium is particulate in nature. He was enabled to show that whatever the degree of dilution, the local effect produced (provided that it is produced at all) is always the same. In other words, the difference between the effect of inoculating extremely diluted lymph, and that of inoculating the lymph itself without any addition of water, does not manifest itself, as might be expected, in the size of the individual vesicle or vesicles produced. He found, on the other hand, that the difference consisted entirely in the numerical relation of successes to failures. If success-

ful, inoculation with a liquid containing an extremely small proportion of lymph may communicate the disease as completely as the original lymph itself, a result which is not only perfectly consistent with the fact that contagium is particulate, but which is in itself a necessary consequence of its being so.

As bearing on this question, it may be mentioned that Schultz of Berlin has quite recently been engaged on an investigation, having for its object the determination of the extent to which, in order to save time and expense, glycerinated vaccine lymph could be diluted without losing its efficiency as vaccine. In the course of his experiments he found that an emulsion containing one part only of vaccine pulp in two thousand parts of glycerine and water, was capable of producing locally a normal vesicle in a small proportion of the cases in which it was employed for vaccination.

The experiments of Reiter confirmed those of Chauveau, and showed that vaccine lymph, even when excessively diluted, was capable of affording positive result on vaccination, provided the more extensive the dilution the greater the absorbing surface.

In 1872 a paper was published by Cohn of Breslau, in which he treated the morphological aspects of the subject with much completeness. His observations, which related to both vaccine lymph and variolous lymph, have received general corroboration from all subsequent workers. He, however, apparently believed certain micro-organisms found by him to be of one species only, to which accordingly he gave the name

micrococcus vaccinæ or variolæ, as the case might be, whereas later observers have shown that organisms of more than one species are usually to be found in any given specimen of crude lymph. Cohn called attention to the fact that in perfectly fresh vaccine lymph the "corpuscles" for the most part occur singly, but that others are joined together in pairs in a form resembling the figure 8; and he states that after the lymph has been kept for a time the number of the "double cells increase, and soon chains of four begin to be distinguishable. These chains are usually curved or in zigzags; their attachment one to another is evidently very slight, as they can readily be displaced. . . . After a few hours' observation they are seen to be all aggregated into irregular colonies or clumps, each consisting of sixteen, thirty-two, or more corpuscles." He also noted a point of importance in connection with the opacity which is apt to occur in stored lymph, namely, that "in capillary glass tubes the enlargement of colonies sometimes lasts a long time, so that groups of them acquire considerable size, and present themselves as flocculi."

Klebs (1873) isolated from vaccine lymph micrococci united together in the form of tetrads, which he considered as specific, and to which he gave the name of tetracoccus vaccinæ. Cornil and Babes (1883) also met with micrococci. Koch (1882) found micrococci in the vaccine vesicles of a child, and noted that human lymph, freshly prepared with glycerine, contained what he appears to have regarded as the specific bacteria of vaccine. These developed but slowly, even in the

incubator, and were met with in fewer numbers in old lymph, even though it still remained active.

Quist in the following year (1883) published a series of experiments dealing with the possibility of cultivating, outside the animal body, the micro-organisms present in vaccine lymph. The culture fluid employed by him was composed of equal parts of blood-serum, glycerine, and distilled water, this mixture being rendered alkaline by the addition of $\frac{1}{300}$th part of carbonate of potash. After sterilising this fluid by exposing it to a temperature of 60° C. for one and a half hours on three successive occasions, it was inoculated with a minute piece of sterilised sponge soaked in clear lymph, or with a piece of vaccine "crust," which had been washed in distilled water and then carefully dried. He found that growth eventually occurred both on and below the surface, the former consisting of minute scales, while the latter gradually settled to the bottom of the vessel as a fine sediment. The scales, forming a scum on the surface of the fluid, he found to be composed of swarms of micrococci, which, when inoculated on the skin of calves, in some cases gave rise, in his opinion, to a typical eruption of vaccinia. Although Quist did not attain any great measure of success in the immediate object of his inquiry (the identification of the vaccine organism), still he showed that the specific contagium of vaccinia could exist for a time, at any rate, in a fluid containing a considerable portion of glycerine.

About this same time papers were published by Feiler (1883), Serebriakoff (1884), Baragi (1884), and

Hubert (1884), all of whom denied that vaccine lymph contained any specific microbe whatever. Voigt isolated three species of bacteria by making gelatin plate cultures of vaccine lymph. Of these one, which alone was constantly to be found, he considered to be specific, and consequently termed it the vaccinococcus. He describes this as a small coccus growing usually in groups of two or four, sometimes in masses, and, less often, in chains. These bacteria gave rise to glistening white circular colonies, which did not liquefy the gelatin in which they were grown. He further stated that cultivations of this bacterium, when inoculated on calves, rendered them immune to subsequent vaccination.

Guttman (1886) also found micrococci in the contents of vaccine vesicles. These were the micrococcus pyogenes aureus, micrococcus pyogenes albus, and micrococcus viridis flavescens, but none of these is specific to vaccinia.

Marotla (1886) found a coccus (micrococcus tetragonus) which he regarded as specific. By its use he thought that he had succeeded in producing vaccine vesicles. From the characters which he assigns to his cultivations, it would appear that in reality what he isolated was the staphylococcus pyogenes aureus.

In 1886 Meguin called attention to the fact that the number of micrococci contained in vaccine lymph increased considerably from the second day onwards after the removal of the lymph.

During the same year (1886) Dr. Buist succeeded in isolating from vaccine lymph three varieties of micrococci,

which, when grown on nutrient media, gave rise to colonies of a white, yellow, or orange colour respectively. All these Buist appears to have considered to be essential constituents of vaccine lymph, as is evidenced by the fact that he speaks of them as white, yellow, and orange vaccine respectively. From specimens of variolous lymph he succeeded in obtaining one organism only, the colour of which was white when grown on solid media. With none of these cultures did he obtain any definite result on the inoculation of calves, monkeys, or human beings.

A certain small measure of success appears to have attended inoculation experiments in the hands of Carmichael (1887), who does not, however, give any detailed description of the morphological characteristics of the growths which he obtained, and which, by the way, although differing one from another in colour, he seems to have considered were the product of one species of micrococcus only. As was doubtless the case with Quist's experiments, in the few instances in which true vaccine vesicles resulted at the site of his inoculations, success was in all probability due to the presence in the material employed of a small proportion of the lymph originally used for the seeding of his culture medium.

Tenholt (1887) appears to have isolated from specimens of vaccine lymph a dozen different micrococci, two bacilli, and two yeasts. Hlava (1887), on the other hand, isolated from vaccine vesicles the streptococcus pyogenes, the staphylococcus pyogenes, the staphylo-

coccus pyogenes albus, the staphylococcus cereus albus, and the micrococcus viridis flavescens. Garré (1887) reported the existence in vaccine lymph of two bacilli and of a small coccus, which he considered to be specific; this, when inoculated on the calf, produced the vaccinal eruption, followed by immunity, while in the human being it afforded immunity, although giving rise to no eruption.

Pfeiffer (1887) recorded the existence in vaccine lymph of micro-organisms of several different forms, including a yeast (named by him sachromyces vaccinæ), two sarcinæ (lutea and aurantiaca), a short bacillus allied to proteus vulgaris, but differing from it in not being pathogenic, and certain micrococci, of which one, the staphylococcus cereus albus (Passet), was of constant occurrence, while the staphylococcus pyogenes aureus and S. albus were less frequently met with. Pfeiffer, however, believes that the specific agent of variola and vaccinia is not to be sought for among the bacteria, but rather belongs to the class of sporozoa. This subject will require further notice later on. In addition to the staphylococcus aureus and albus, M. Grigorieu (1889) describes a micrococcus vaccinæ as specific to the disease. This microbe usually develops as a diplococcus, rarely forming chains, and grows slowly on gelatin, which it liquefies after four to six weeks. On the calf it produced a papular eruption, which apparently afforded immunity to subsequent vaccination.

M. Woitow (1890) succeeded in isolating four staphylo-

cocci—aureus, albus, cereus, and citreus. He affirms that the inoculation of a mixture of these microbes produced typical vaccinia in the calf. Professor Leoni (1890) met with several pyogenic microbes in fresh vaccine lymph, notably the staphylococcus pyogenes albus, which was invariably present. M. Protopopoff (1890) in like manner found it possible to isolate several different forms of micrococci.

As the result of my own work previously to 1891, in which year my first paper was published, it would appear that there are at least three species of micro-organisms, namely, staphylococcus albus, staphylococcus pyogenes aureus, and staphylococcus cereus flavus, corresponding probably to Buist's white, orange, and yellow vaccine, respectively, one or more of which are almost universally to be found in every specimen of human or calf vaccine lymph examined. Of these the staphylococcus albus is usually to be found in the upper layers of healthy skin of unvaccinated persons. In addition, I had been able to satisfy myself as to the occasional presence of the streptococcus pyogenes.

I myself have never detected the streptococcus of erysipelas, but Dr. Klein has placed on record the fact that in one instance he isolated this microbe from a specimen of human vaccine lymph, the use of a portion of which was believed to have given rise to the occurrence of this disease. Also I have shown that none of the microbes ordinarily to be isolated from vaccine material can be regarded as specific to vaccinia.

Professor Crookshank (1891) succeeded in isolating

from various specimens of vaccine lymph, by the method of plate cultivation, an immense number of bacteria, including micrococci, bacilli, torulæ, etc. All of these he recognises as well-known saprophytic bacterial forms, associated, some of them, with processes of suppuration, but none of which, he says, can be regarded as the contagium of vaccinia, seeing that no single one of them is constantly present in vaccine lymph, human or bovine.

M. Besser (1893) found in the papules of a case of smallpox, at the fifth day, a small bacillus, which he is disposed to look upon as the specific organism of variola. He obtained cultures on various nutrient media, coverslip preparations from which always showed the organisms arranged in palisade-like rows.

The late Professor Straus (1893) found that the seeding of gelatin plates with fresh vaccine material, whether glycerinated or not, gave rise to the appearance of numerous colonies of micrococci, among which the staphylococcus pyogenes aureus and S. albus were most frequently met with. When glycerinated lymph which had been kept for a considerable period was employed in a similar fashion, the number of colonies was greatly diminished. The paper, in which he collaborated with MM. Chambon and Ménard, is illustrated with three figures of plate cultures from vaccine lymph, which had previously been stored for different periods of time.

M. Anthony (1893), in the course of the examination of a number of different specimens of vaccine lymph, glycerinated and otherwise, found that four different kinds of micrococci and three species of bacilli are apt

to be present. Of these, what he terms, from its appearance, the "porcelain micrococcus," is invariably present in fresh vaccine. The other three micrococci included the staphylococcus pyogenes aureus, a grayish-white micrococcus liquefying gelatin, and a yellow micrococcus often present, which does not liquefy gelatin, and which grows easily in peptone beef-broth, on gelatin, and on agar. The four bacilli noted by him include the bacillus subtilis, the bacillus mesentericus, a fluorescing bacillus, and a motile bacillus which grows in the form of yellow colonies. All these bacilli, according to this observer, represent impurities of the lymph. As the result of his researches, M. Anthony concludes that vaccine lymph cannot be regarded as above suspicion so long as it is found to contain any microbes other than the porcelain micrococcus. The inoculation of cultures of his porcelain bacillus on heifers afforded only negative results.

M. Maljean (1893) published a paper in which he describes the occurrence in vaccine lymph of two yellow cocci, of which one liquefies gelatin and the other does not, and of a peculiar micrococcus which is of a brilliant white colour when grown on various media, and to which he gives the name of "*coccus vaccinal.*" This micrococcus is larger than streptococcus pyogenes, and in cultures it grows as isolated points, as diplococci, and in short chains. Maljean states that this coccus gave rise, in the guinea-pig, to the formation of a suppuration, and on the calf to a typical vaccine eruption. Other observers, however, employing the same cultures, failed to obtain a positive result.

Dr. Klein agrees with me in maintaining that none of those microbes, which ordinarily can be cultivated from vaccine lymph, are "an essential inhabitant in vaccine" or "can have anything to do with its active principle." In the Report of the Medical Officer to the Local Government Board for 1892-93, he, however, described a peculiar, extremely minute bacillus as occurring in calf lymph, and in variolous lymph of human origin, obtained at an early phase of the disorder; in calf lymph 72 to 96 hours after vaccination, in human variola during the third or fourth day. In both instances the lymph was collected aseptically. Clear lymph only, as free as possible from epidermal débris, was used for film specimens, which, after heating and treatment with 30 per cent acetic acid for some minutes, was subjected to prolonged staining in alcoholic gentian violet. Some of the films of calf lymph (collected after removal of the epidermis as a whole) showed an abundance of these minute bacilli, generally massed together, some of the specimens looking indeed like film specimens of an artificial culture. Lymph of early human variolous vesicles showed similar bacilli, but not so abundantly. Calf lymph taken at later stages (five or six days old) showed no bacilli or only a few here and there. In certain of these bacilli from vaccine lymph, Dr. Klein succeeded in demonstrating spore-like bodies which, together with the absence of the bacilli in the lymph of later stages, led him to the conclusion that disappearance of these bacilli from late lymph was possibly the result of their having broken up after the

formation of spores, which might alone be present about the time of maturation of the vesicle. Dr. Klein adds that these bacilli are incapable of cultivation in the ordinary culture media, no growth whatever having been obtained by him on solidified blood-serum, glycerin agar, ordinary agar, sugar gelatin, or ordinary gelatin, although in specimens of the same lymph as was employed for this purpose, and which on transference to the calf produced perfect vaccine vesicles, bacilli were found in abundance.

MM. Baillard and Anthony (1894) have worked at the question of the number of microbes present in vaccine which has been stored for various periods. Baillard constantly found in the lymph a white staphylococcus, a yellow staphylococcus, and bacillus subtilis. His experiments led him to the conclusion that, by keeping, the number of germs contained in glycerin pulp become considerably diminished, but that germs never completely disappear. After so long a time as seven months the material with which he was experimenting still contained some living germs, among which were the bacillus subtilis and staphylococcus albus. Leoni (1894), also experimenting on the effect of glycerin in the purification of vaccine lymph, found that the microbes usually present had disappeared in from one to four months.

Dr. Buttersack (1894) described the appearance in vaccine lymph, dried on cover-glasses, of a dense network of threads associated with numerous granules, which he considered as peculiar to vaccine. These threads he regarded as the growing vegetative part of

a vaccine fungus, the granules in his opinion representing spores. It appears fairly certain, however, that what he believed to be a mycelium with spores, in reality consisted merely of threads and granules of fibrin or of some other albuminous substance.

In this same year (1894) I published a description of numerous minute bacilli sometimes to be found in considerable numbers in the vaccine vesicles both of the calf and of the human being at an early stage of their development, in this respect, apparently, confirming the experience of Klein, which was published in the same year, and of which mention has already been made. Similar bacilli were also demonstrated by Mr. Kent and myself in sections of skin passing through the site of a vaccine vesicle obtained from the calf.

An account of certain experiments dealing with the possibility of cultivating these micro-organisms, and also a similar one isolated from variolous material, I propose referring to later.

M. Ruete (1894) obtained what he believed to be pure cultures of the specific agent of vaccine from calf lymph, this consisting of a micrococcus, the toxin elaborated by which he termed vaccinin. He failed, however, in his attempts at inoculation of the human being.

In 1895 Landmann found that in Germany no less than 80 per cent of certain children vaccinated successfully presented an excessive inflammatory condition of the arm, sometimes of an erysipelatous or hæmorrhagic character. In searching for the cause of these unfortunately numerous complications, Landmann undertook an

elaborate study of the lymphs distributed from thirteen of the German institutes. As a result of his research, he found that the number of germs present in the various specimens of lymph varied from 50 to no less a number than 2,500,000 per cubic centimetre. Among the microbes which he succeeded in isolating were the streptococcus pyogenes, the staphylococcus albus, and the staphylococcus aureus.

Two American observers, Dr. Stephen C. Martin and Professor Ernst, recorded in 1895 the isolation of a micro-organism which they considered to be specific to vaccinia. This was a short thin bacillus, which they found was capable of growth on serum obtained from ox or horse's blood, but not on other media. The growth, which is of a white colour, forms chains, and, under certain circumstances, according to these observers, takes on the form of a micrococcus. With a cultivation of this microbe of the fourteenth generation they produced a vaccine vesicle on an infant in one attempt out of ten; better success, however, attending their attempts at inoculation of a calf. M. Le Dantec in the same year (1895) found, in lymph, staphylococci, the reactions of which, he says, show that they are related to the ordinary pus cocci. These staphylococci differed according to the species of animal from which they were obtained; those isolated from calf lymph liquefy serum, while those obtained from human lymph did not do so.

M. Arloing (1896) experimented with the lymph of vesicles of horse-pox, which he had obtained by injecting

a colt intravenously with vaccine lymph purified as far as possible by storage in glycerin. From the resulting vesicles he first carefully removed the superficial layer of epidermis, and then collected the underlying material with aseptic precautions. With this he inoculated tubes of glycerin beef-broth, obtaining as a result, in each instance, a growth consisting of a micrococcus, which, however, when inoculated in turn on the animal body, produced neither an eruption nor immunity to subsequent vaccination. From his researches Arloing concluded that the virulent agent of vaccinia must be of the nature of a soluble toxin.

MM. Boureau and Chaumier, also in 1896, found a number of different microbes in specimens of vaccine lymph, including staphylococcus aureus, cereus, and albus, micrococcus flavus, bacillus subtilis, and luteus, bacterium termo, proteus vulgaris, a cladothrix, a fluorescing bacillus, and several other bacilli which they were unable to identify. They make the extraordinary statement that it is only when the vaccine has lost its virulence that the microbes originally contained in it completely disappear; that in fact the number of staphylococci present at any given time afford a measure of the potency of the lymph.

Dr. Saint-Yves Ménard (1896), in reply to this communication, reiterated the contention of Straus and himself, confirming my previous work on this subject, that the microbes which can be isolated from vaccine lymph by the ordinary methods are in no way essential to its specific action, and that by the end of six months,

at any rate, no micrococci can be obtained from "glycerinated emulsion," which has been preserved in sealed tubes, although it is still perfectly active as vaccine.

Dr. Sacquépée (1896), in the course of examinations of various samples of vaccine lymph, met with three species of staphylococcus, the bacillus subtilis, the bacillus mesentericus, and another unknown species of bacillus. Of these he regards the bacillus subtilis and the bacillus mesentericus as evidence of accidental impurity of the lymph. Delobel and Cozette (1896-97) collaborating with Gourny, in common with most other observers, found that the bacteria most constantly present in vaccine lymph are a white and yellow micrococcus. They state that when due care is not exercised in the collection of the lymph, bacillus subtilis and bacillus mesentericus may be found. Inoculation experiments on the human being and on the calf afforded them only negative results.

M. Paul, also in 1896, published a voluminous account of his researches on the occurrence and significance of pathogenic micro-organisms associated with vaccine lymph. He lays stress on his observation that the staphylococcus pyogenes aureus is by far the most ubiquitous of the microbes that are found in vaccine lymph, although it is certainly not an essential concomitant. He finds, moreover, that it exhibits both variable resistance and variable virulence. A sample of lymph, for instance, of supposed Jennerian stock he found to contain this staphylococcus in a phase of much higher virulence and

resistance than was a specimen of this same microbe obtained from a sample of calf vaccine lymph. This staphylococcus, he adds, may be present without causing suppuration; but the use of a lymph containing it in large quantities should, naturally, be avoided. According to this observer, the number of bacteria present in fresh lymph appears to be independent both of the origin of the stock from which it is derived and of the method of vaccination employed. This, as shown elsewhere, does not accord with my experience.

Quite recently (1897), in conjunction with Dr. Blaxall, I have carried out a further exhaustive series of investigations on the bacterial flora of calf vaccine, with the outcome that, as the results of former work had led us to anticipate, and, as has been repeatedly shown by other observers, calf lymph (or rather vesicle pulp) may contain a large number of micro-organisms which are in no way concerned with its specific activity. Human lymph, as a rule, we find to contain remarkably few microbes, but, probably, this might not be the case were the whole substance of the vesicle removed as is now usual in collecting from the calf. We have shown also, as have other investigators, that lymph, if carelessly collected, is often found to be contaminated with many of the numerous saprophytes common to dust, etc., in addition to certain possibly pathogenic microbes which may be present. In the particular series of experiments here referred to, and in a very large number of examinations of samples of calf lymph, we have met with the following micro-organisms at one or another time.

They are arranged in the order of their prevalence and predominance.

1. Staphylococcus cereus flavus
   „ „ albus (Passet.)
2. Large yeast, orange coloured.
   Small „ light-brown colour.
   Small „ pale salmon colour, and very slow growing.
3. Staphylococcus pyogenes albus (Rosenbach.)
4. „ „ aureus (Rosenbach.)
5. „ „ citreus (Passet.)
6. Bacillus mesentericus vulgatus.
7. „ subtilis.
8. Moulds, *penicillia*, *mucors*, *aspergilli*: Sarcinæ, *lutea*, *aurantiaca*.

In the series of experiments here dealt with, one or more members of groups 1 and 2 were always present. The slow-growing yeast was not, however, generally visible as a distinct colony till the seventh or eighth day.

Staphylococcus pyogenes albus was frequently present in lymph, but generally in small numbers. Staphylococcus aureus was rather less frequently observed, and staphylococcus citreus was only occasionally met with.

Bacillus mesentericus may be regarded as an accidental contamination.

Bacillus subtilis rarely occurred if precautions were taken; its presence seeming to depend entirely on the care exercised in cleansing the skin, and in collecting the lymph material.

The same is true of group 8.

It must be stated, however, that all the samples of calf vaccine examined were derived from the same station, namely, the Government Animal Vaccine Establishment, Lamb's Conduit Street. Here, calf-to-calf vaccination is continuously practised; so that the adventitious saprophytes found in the lymph, at one and another time, are apt to be similar in kind, the extraneous organisms originally present having been, so to speak, cultivated *pari passu* with the vaccine virus itself. Very probably, therefore, a strain of lymph from another station might present a "flora" differing in kind and in amount, as, indeed, has been found to be the case with certain foreign lymphs which we have had the opportunity of examining.

On the other hand, lymph taken directly from calves vaccinated with vaccine material rendered free from extraneous organisms, as for instance glycerinated lymph a month after emulsification, often presents remarkably few colonies of "extraneous" organisms. In one such sample only four colonies appeared on a plate-culture of the lymph; one of staphylococcus cereus albus, and three of a light-brown yeast.

If a vaccine vesicle of a healthy child or calf be carefully opened at an early stage with all aseptic precautions, and a droplet of the lymph which exudes from it be submitted to bacteriological examination, it may not unfrequently be found, as Klein has also demonstrated, that such lymph is absolutely pure in the sense that it contains no micro-organisms which will grow on ordinary culture media. Yet it is perfectly efficient as vaccine.

But, on the other hand, as just stated, in lymph obtained in ordinary fashion from mature vesicles, inoculations of plates or tubes of nutrient material usually result in abundant growths of micro-organisms. From the list of species which I have set out, it will be seen that those which are most commonly met with are, morphologically and culturally, identical with the staphylococci found in pus, viz., the staphylococcus aureus, albus, cereus albus, and cereus flavus. Of these the first two liquefy gelatin, the other two do not. Occasionally the streptococcus of pus may be present, as also the staphylococcus citreus. Other microbes of a purely saprophytic nature that are met with, include the common hay bacillus, bacillus mesentericus, varieties of proteus, yeasts, moulds, and sarcinæ. These, however, can for the most part be excluded by careful manipulation, so that in vesicle pulp, which has been collected with all due precautions, nothing is found beyond some one or more of the four first-named staphylococci, of which a white staphylococcus is perhaps most common, and possibly one or more yeasts. Thus our most recent experience confirms in all essential particulars my first report published in 1891.

The fact that in vaccine lymph or in the vesicle pulp micro-organisms are found which, under certain circumstances, are known to be provocative of suppuration, does not in any way imply that the lymph in which they are present is purulent, or that, if such staphylococcus infected lymph were employed for vaccination in the human being, suppuration would necessarily ensue. The

recent Berlin Commission, indeed, report that of eighteen samples of lymph examined only five contained staphylococci which were pathogenic for small animals, and, further, that no harm resulted on the vaccination of children with these particular specimens of lymph. From this circumstance it is evident that the virulence of these micro-organisms for rodents, for instance, cannot be accepted as a measure of their power to affect injuriously the body tissues in the healthy human subject. The streptococcus of pus must be a rare inhabitant of calf lymph, the Berlin Commissioners indeed stating that they never once met with it in the course of the examination of sixty-four samples of lymph. In human lymph its occurrence is by no means so unfrequent.

With reference to the question of the possible presence of the tubercle bacillus in lymph, I am not aware of any authentic record of its having been found, even in lymph derived from the calf. But, as I shall presently have occasion to show, we are enabled by the use of a special method of preparing and testing vaccine lymph to obviate any possibility of danger to which the presence of the tubercle bacillus or other pathogenic microbes in the original specimen of lymph might conceivably give rise.

In 1894, as already mentioned, I showed concurrently with Dr. Klein, though independently, that in specially-stained specimens of vaccine lymph taken at a period antecedent to full maturity of the vesicles, the presence of bacilli of extremely small size and in practically pure

culture can be demonstrated. These bacilli cannot be found, or only with difficulty, in mature lymph, for the reason probably that they have by then given place to spores. For some time it appeared impossible to obtain further evidence as to the *rôle* of these bacilli, as they altogether refused to grow on any of the ordinary culture media, and under either aërobic or anaërobic conditions, though this very fact obviously tends to show that they are not of a merely saprophytic nature.

In the following year, however, I carried out certain experiments which appear to prove that it is possible to obtain a growth of similar bacilli from variolous material in a particular culture medium, and even to carry on such growth in secondary cultivation. The experiments which have been instituted in this direction are but few in number, and it is evident that the exact conditions essential to success have yet to be determined.

As it seemed to me, the desideratum for the purpose of inducing growth of the supposititious micro-organisms was a medium which had not been artificially sterilised, which *à priori* should afford the necessary pabulum, and which at the same time was secure in its circumstances against contamination. Hence, the choice appeared to lie between the intraperitoneal fluids of one or another living animal and the hen's egg, which has already been made use of as an alternative culture medium. As regards intraperitoneal fluid I foresaw difficulty, in that it is apt to contain leucocytes, which might easily vitiate an experiment, and accordingly I adopted the hen's egg as matter of first instance. I

had no experience to guide me as to the best method of carrying out the inoculation of the egg, or as to the period of incubation best suited for the object I had in view, and my attempts for a time, therefore, met with but small success.

My inoculations were carried out by first cleansing the surface of the egg with corrosive sublimate solution and afterwards with alcohol, and then breaking a small hole in the shell by means of a sterilised needle and forceps. Through the opening thus made I mixed the yolk and albumen of the egg by means of a platinum needle, and then carried out the inoculation by means of a glass capillary pipette, which had recently been drawn out in the blow-pipe flame.

For the purpose of such inoculations I employed variolous crusts, obtained from the smallpox ships of the Metropolitan Asylums Board. These crusts were rubbed up in a small glass mortar with a minimal quantity of water or sterilised normal saline solution. Of the resulting emulsion a drop or two was then taken up into the glass pipette and the inoculation carried out. Finally, the small hole in the egg was closed up, either by means of a small plegget of sterilised cotton-wool soaked in collodion or with melted sealing-wax.

The eggs inoculated in this manner were then placed on beds of cotton-wool, and kept in the incubator at a temperature of 37° C. Here they were left for varying periods of time, a month being the period which was eventually found to be that which was most desirable.

On opening an egg thus treated, I found that in its

interior was contained a creamy material, which in great part had replaced the ordinary egg contents. This material, examined in cover-glass specimens, appeared to contain a pure culture of one organism only, namely, a bacillus which morphologically was not to be distinguished from the bacillus which I have previously described as to be found in early vaccine lymph.

My next step was to attempt to grow it in sub-culture, and to make observation also of the effect of its direct transference by cutaneous inoculation into the calf. As regards attempts at sub-culture in all media most usually employed for such purpose, including gelatin, sugar-gelatin, agar, glycerin-agar, sugar-agar, and serum, these at this time failed, as sub-culture of the bacilli contained in vaccine lymph had always failed when the ordinary culture media are adopted. But with the calf it was different, and, as the result of inoculation of egg-cultures on this animal, I obtained a strain of lymph which, after being passed through a series of calves, was successfully employed for the vaccination of children. The calves were all subsequently vaccinated with negative results.

The method of experimentation was as follows :—

Experiment 1.—On 26th March 1895, calf A was inoculated by eighteen incisions (fifteen on the abdomen and three on the scrotum), with a small quantity of the creamy emulsion contained in egg No. 5, which had been incubated for one month after inoculation with smallpox crusts, as already described. On 30th March, scrapings were taken from the vesicular points

which had appeared in the line of two of the abdominal incisions, and also from one of the incisions on the scrotum, which had become slightly raised and red. With material thus obtained, calf B was on the same day inoculated by six linear incisions on a specially shaved area on the abdomen. Three incisions were inoculated with scrapings taken from the vesicular points, and three with scrapings taken from the incision on the scrotum of calf A. Three days later (on 2nd April) all six insertions appeared to be taking, though eventually one died away. On 4th April the calf was photographed, and on the same day a child was vaccinated from it by Dr. Cory. This child I have since seen. It has five good marks, and the mother told me that the vaccination "did splendidly," and that she had had no trouble with the child. From calf G, calf H was inoculated on 25th May; and, further, this strain of variola-vaccine, derived in the first instance from the egg culture, was carried on through two additional removes in the calf, after which it was not further cultivated.

Eight calves in all were inoculated in this manner, and on 28th May 1895, lymph taken from vesicles at the third remove from the egg culture was, in my absence, employed for the vaccination of a large number of children at the Animal Vaccine Establishment, owing to the fact that for some reason or other the current strain of lymph had failed to give good results in the two calves which had been vaccinated according to the usual routine. All these children

showed excellent marks when inspected a week later, and several of them were photographed by my colleague, Mr. Kent.

Interesting, and indeed satisfactory, as are these experiments from my point of view, I do not claim for them demonstration of the practicability of growing and multiplying outside the animal body, in artificial media, the particulate cause of smallpox, and of converting the latter by my processes into the essential cause of vaccinia. And for this reason: these experiments of mine on the bovine animal had to be conducted—at any rate, in this preliminary stage—at the Animal Vaccine Establishment in Lamb's Conduit Street, and the subjects of them had to be calves that on each occasion of experiment by me were at the same time vaccinated in some part of their bodies with the calf lymph current at this station. It is true that every precaution was taken in the way of sterilising instruments and cleansing operation tables; in selecting a given and clearly defined area on each animal for insertion of my lymph; and for keeping the incisions made by me protected, as far as possible, against later accidental mediate inoculation with lymph from some part of the same or other calf into which Animal Vaccine Establishment material had been inserted. Nevertheless, it is possible that, in spite of all precautions, accidental inoculation of my incisions with Animal Vaccine Establishment lymph may have occurred; and, further, I am not able to deny that the fact of simultaneous inoculation of the calves with my

egg culture and with ordinary vaccine lymph may not have, in some occult way, influenced the manifestation witnessed on the areas wherein my egg lymph was inserted. So, of course, these experiments have to be repeated and are, indeed, in course of repetition.

More recently, Dr. Blaxall and I have been further investigating the possibility of the cultivation in artificial media of the micro-organism specific to smallpox and vaccinia respectively. Our work received considerable impetus by the discovery that certain culture-tubes, which had been inoculated with vaccine lymph, but which appeared to remain completely sterile, nevertheless contained a growth of a microbe indistinguishable morphologically from that which I found in egg cultures. This growth, although invisible to the naked eye, became obvious when cover-glass impressions were taken from the surface of the nutrient medium and afterwards stained and examined with the microscope. Dr. Blaxall subsequently devised a method by means of which pure cultivations of apparently the same organism were obtained on the surface of agar plates, and this not only when vaccine lymph, both of human and bovine origin, was employed, but from variolous lymph as well. From these plates agar tubes and tubes of peptone water were also in turn inoculated. In these again, in the first removes, practically no growth was visible, except on careful examination by reflected light; but as generations further removed from the original stock were obtained, the growth became more obvious, showing as a haziness in the fluid media, and

as a semi-translucent grayish-white line on a sloping surface of agar. Broth cultures of the third and fourth generations, when employed for the inoculation of a calf, afforded results which we regarded as most encouraging, seeing that we were enabled to carry on the infection to other calves and subsequently to children, in all of which it took the form of typical vaccinia. Some weeks after, the calves were vaccinated with active calf lymph, but without result. A later series of experiments, however, was not attended with similar success. The reasons for so great a divergence in our results at one and another time are by no means obvious, unless possibly the fact of increased vegetative function shown in the later sub-cultures of the microbe may have had as a concomitant a lessened degree of pathogenicity. On this point we are, at present, unable to make any definite statement, but, needless to say, the investigation is being continued.

Another direction in which our research work has been extended was suggested to us by certain statements in the "Report of the Berlin Scientific Commission." In that report an account is given of certain experimental inoculations performed by Freyer with the juice of the spleen, liver, inguinal and mesenteric glands, and marrow of previously vaccinated calves. Using such material, vesicles were produced by the employment of almost every organ, which shows that, in Freyer's opinion, the vaccine germ must be conveyed to them by the circulating blood. This general distribution of that micro-organism takes place,

he states, up to three or four weeks after vaccination, but no mention is made by him as to the earliest period at which it was found to occur. The greatest amount of success was obtained by the use of inguinal gland and spleen juice. He further stated that since calves were successfully vaccinated with this material, the postulate of an absolutely bacteria-free efficacious lymph was complied with.

It is, of course, obvious that where generalised vaccinia occurs as the result of a local vaccination as, according to Chauveau, is always the case with solipeds, the micro-organism concerned must have been conveyed to a distance from the original point of insertion by means of the circulation. Presumably this may also take place, although perhaps to a more limited extent, in that form of the disease in which, as in the case of the vaccination of the human being or the calf, the resulting disorder remains to all appearance strictly localised, a secondary eruption being prevented by the spleen or other organ, such as the liver, being enabled to seize upon and ultimately destroy any of the specific germs that happen to be carried thither.

It seemed to me that if Freyer's results were capable of confirmation, the possibility of vaccinating by means of spleen-juice, for instance, must depend on the employment of such material at a period sufficiently late to ensure the active principle having reached the particular organ, and antecedent to that at which it would have been destroyed. I thought also that, if the most favourable period could be determined, it

would be possible then to obtain a material free from bacterial growth other than that on which its potency for vaccination depended—a material, therefore, which would afford exceptionally favourable opportunity for the isolation of the organism specific to vaccinia. But, unfortunately, notwithstanding the categorical statements in the Report of the Berlin Commission, we have been unable, as the outcome of some preliminary experiments, to obtain results comparable to those described by Freyer. Neither have we been able to demonstrate the presence of a specific micro-organism in culture media inoculated from the fresh spleen, liver, or glands, obtained at various dates after vaccination, or in the interior of fresh or hardened sections of these same organs.

Protozoa

Owing no doubt to the fact that hitherto it has appeared practically impossible to isolate from the lymph of vaccinia or variola any bacterium which could with reason be regarded as specific to these affections, a number of observers have turned their attention to the search for a micro-organism of somewhat higher type, which might by chance be concerned in the dissemination of vaccinia and variola respectively. Among the first experimenters in this direction must be mentioned Renault of Lyons, in 1881; Van der Loeff in 1886, and Hlava and Pfeiffer in 1887. It was not, however, until the publication in 1892 of Guarnieri's work that interest in the problem was seriously aroused. Since

then the literature of the subject has expanded enormously, owing mainly to the labours of numerous Continental pathologists, the only English contributions to the mass of experimental work published up to the present, being those of Drs. Ruffer and Plimmer and of Mr. Jackson Clarke.

Renault (1881) first called attention to the fact that peculiar bodies, which he regarded as parasitic, could be demonstrated in the epithelial cells of the vesicles in vaccinia and variola. Van der Loeff, also, on examining in the warm chamber a hanging drop of clear calf vaccine lymph, discovered "numerous small bodies capable of independent movement," of which, however, he gives no further description; although he offered the opinion that they were living organisms which should be classified with the Rhizopoda.

In 1887 L. Pfeiffer described the occurrence in lymph, obtained from cases of vaccinia and variola, of small, unicellular, generally spherical bodies, which he believed to constitute the specific contagium of these diseases. These parasites he found in still greater numbers in the Malpighian layer of the skin, multiplication taking place by division and also by endogenous formation of spores. These statements were in part confirmed immediately afterwards by Rieck.

Guarnieri having invariably found, in the epithelial cells of the skin in the vesicular stage of smallpox, small particles half the size of the cell-nucleus or even smaller, next attempted (in 1892) the cultivation of similar bodies in the living animal. For this purpose he inoculated the

cornea of rabbits and guinea-pigs with vaccine lymph, with the result that these protozoa, as he considered them, appeared in the cells of the epithelium covering the cornea. After an interval of about fifty hours from the time of inoculation, the surface of the cornea was scraped, and the material examined in a hanging drop of aqueous humour. Guarnieri also employed for examination sections taken through the site of inoculation and stained in various ways. In either case he found numerous "parasites" within the epithelial cells of the affected area, generally occupying a zone of the protoplasm immediately surrounding the nucleus but not intruding upon it. In the hanging drop they appeared as little refractile bodies endowed with slight amœboid power. Examination of sections further showed that they vary much in shape and size, and, further, that they are most numerous in the deep layers of the epithelium, the superficial cells not being involved. To this "parasite," Guarnieri gave the name of Cytoryctes vaccinæ. Monti obtained similar results, and both Guarnieri and Monti exhibited, at the International Congress of Medicine at Rome, preparations in which they demonstrated the presence of the parasite. Messrs. Ruffer and Plimmer, who in this country had been working at the subject, also attended the Congress, and were thus able to compare their own specimens with those shown by Guarnieri and Monti, thereby establishing, according to Dr. Ruffer, the fact that the protozoa described by all of them were identical in character.

Messrs. Ruffer and Plimmer, writing in 1894, described the alleged parasite as a small, round body, which, when treated with appropriate reagents, appears to have a more darkly staining centre. It is, they say, about four times the size of an ordinary staphylococcus, and generally lies in a clear vacuole in the protoplasm of the epithelial cells of the stratum Malpighii, and occasionally indents the nucleus, although they have not found it enclosed in the latter body. These observers state that they have found the same organism in the sections of skin from smallpox patients and in smallpox pustules of the larynx and the trachea.

In the same year (1894), L. Pfeiffer not only confirmed the results obtained by Guarnieri, but announced also the discovery in the blood of persons suffering from smallpox and in the blood of children and calves that had been vaccinated, of amœboid cells which he regarded as parasites. These he found to be most numerous during the pyrexial stage of the disorder, whether variola or vaccinia. These bodies, which are somewhat larger in the blood of the calf than in that of the child, being in the latter about a quarter the size of an average blood corpuscle, are, Pfeiffer states, provided with pseudopodia and are occasionally flagellated. According to this observer they also contain from one to four nuclei, which are readily stained. They occur free in the blood, not being contained in cells as is the case in the vaccinated cornea; nevertheless, Pfeiffer considers that they represent a similar stage in the life history of the organism, of which the more mature form

is of a cystic nature, to which he gives the name Monocystis epithelialis. This cyst he believes to give rise, in due course, to a number of spores. It should be mentioned that he describes similar bodies as occurring in various other vesicular eruptions of man and the lower animals.

A Japanese observer, Ogata (1895), as the result of careful microscopic examination of stained film-preparations of vaccine lymph, derived from the human being and the calf, and also of human variolous lymph, concludes that certain protozoa, which he has been able to demonstrate in each case, should be classified with the Gregarinidæ.

Further confirmation of Guarnieri's experiments was afforded in 1895 by Von Sicherer, Ernst Pfeiffer, and Mr. Jackson Clarke. According to E. Pfeiffer the bodies vary considerably both in size and shape, being sometimes as large as a red blood-corpuscle. They resemble the corpuscles also in their affinity for certain staining reagents.

There can, of course, be no doubt as to the occurrence of most of the appearances described, although we may not be prepared to accept the interpretation put upon them; and in this connection it is well to bear in mind not only that the bodies enclosed in cells, described by Guarnieri, Monti, and Ruffer, are said by them to differ essentially in their staining reaction and in their appearance from those of the Pfeiffers, Van der Loeff, and others, but also that all the more recent experimental work in this direction has been carried out on the cornea

of the rabbit and the guinea-pig, both of them animals which there is reason to believe are insusceptible to vaccinia. It is therefore, I think, conceivable that the "parasites" in question may represent merely the result of epithelial irritation caused by the scarification, together with that—of a non-specific nature, however, —set up by the vaccine lymph employed.

That such is indeed the case there is now a considerable amount of evidence to show. Thus Ferroni and Massari (1893) expressed the belief that Guarnieri's so-called parasites are in reality derived from the nuclei of the cells, or are due to the immigration of leucocytes. They base their opinion on the results of certain experiments devised by them, in which they inoculated the cornea of rabbits and guinea-pigs with croton oil and India ink. On subsequent examination of stained sections, they were able to demonstrate the presence in the epithelium cells of numbers of small bodies apparently identical with those described by Guarnieri. It should, however, be stated that E. Pfeiffer failed to find them under similar circumstances, although they were invariably present after the inoculation with vaccine lymph.

In the *Annals* of the Pasteur Institute for April 1897 appeared a most important paper by M. Paul Salmon. This investigator brings to bear on the subject a thorough knowledge of modern staining reactions, by the light of which he shows that Guarnieri's parasites, while differing in their affinity for certain stains from either the protoplasm, nucleus, or nucleolus of the epithelial cell, correspond in all respects, as judged by such tests, to the nuclei of

the migratory leucocytes, the chromatin masses of which rapidly break down in the substance of the invaded cells. He sums up the matter in the following words: "Le pseudo-parasite n'est pas une formation endogène; il a donc forcément une origine extra-cellulaire, extra-épithéliale, et *la petite masse de chromatine ne peut avoir qu'une origine; les cellules migratrices.*" The paper is illustrated with drawings showing all stages of the process of invasion of the corneal epithelium by the leucocytes, from that of their first appearance in the underlying tissue, to that of their final dissolution, represented by the presence of minute granules of chromatin only, in the protoplasm of the epithelial cells. No one, I think, comparing this plate with the drawings and descriptions of the corneal "parasites" of Guarnieri and others, can fail to be struck with the truth and completeness of Salmon's explanation of the facts previously observed.

Recent researches have tended to confirm the statement of L. Pfeiffer as to the presence also in the blood of variola and vaccinia, especially during the pyrexial stage, of minute, free, granular amœboid cells. It is, however, doubtful whether it is possible to demonstrate a nucleus. These cells, as already mentioned, are regarded by Pfeiffer as the causative agent of these diseases.

This question has been investigated in America by Dr. Walter Reed (1897), whose attention was directed in the first instance to the microscopical examination of fresh preparations of the blood of vaccinated monkeys and calves, and subsequently to that of vaccinated children and smallpox patients. Of eleven vaccinated

animals (nine monkeys and two calves), whose blood was examined, Reed found in the blood of seven (six monkeys and one calf) "granular amœboid bodies, having a diameter about one-third that of a red blood cell" during the active stage of vaccinia. These bodies on the decline of the local inflammation again disappeared. As showing, however, that these bodies have no causative relationship to vaccinia is the fact that Reed occasionally met with quite similar bodies in the normal blood of healthy children and of monkeys. This same observer states that pale amœboid bodies, containing a few dark pigment-like granules, may also be present in the blood from cases of variola in the human being and the monkey. The blood of vaccinated children and monkeys was found occasionally to contain bodies of like appearance.

Reed does not appear to have known of a paper published in the previous year (1896) by M. F. Müller, an assistant in Nothnagel's clinic in Vienna. Therein Müller gives a description of "small, generally round, colourless granules," which he found constantly present in the freshly-drawn blood, both of healthy persons and of those suffering from various diseases. These granules, which may, he says, be readily distinguished from blood-plates, would appear to be of like origin with the minute granular amœboid bodies originally discovered by Pfeiffer.

Further light has been thrown on this subject by the labours of Stokes and Wegefarth (1897), who carried out a lengthy series of observations in the bacteriological laboratory of the Baltimore Health Department. The conclusions at which they ultimately arrived may be

briefly set out as follows: In the normal blood plasma and serum of man and the lower animals varying numbers of granules, apparently derived from neutrophil and eosinophil leucocytes, are usually to be found. There is reason to believe that these granules play some part in bringing about agglutination and cessation of motility of many pathogenic bacteria and of ultimately destroying them, a property possessed by the normal blood. That this is so, is judged from the fact that on filtration of dog's or rabbit's serum through a Müncke porcelain filter, this effect on bacteria was no longer exhibited, although the potency of the serum in this direction could be restored by adding to it a sediment containing granules.

Stokes and Wegefarth make also a suggestion which may not impossibly account for the undoubted fact that, as stated both by Pfeiffer and Reed, the minute granular cells described by them are most readily found in the blood during the progress of certain infective diseases, although, as Reed shows, they must also be regarded as normal constituents of the blood. The suggestion referred to is that the bactericidal power of both leucocytes and serum in man and many animals is due to the presence in them of specific granules, derived in either case from leucocytes, which in presence of invading bacteria are given up to surrounding fluids or tissues. As the outcome of the most recent investigations dealing with the question under discussion, it must, I think, be admitted that although Guarnieri and others who have described the presence of protozoa in the blood and tissues of man and the lower animals,

during certain stages of invasion by variola or vaccinia, are undoubtedly correct in their statements as to the occurrence of the particular bodies described by them, they are altogether mistaken as to the true nature and affinities of these bodies. There would indeed appear to be good reason for the belief that these so-called parasites are in fact nothing more nor less than the fragments of amœboid leucocytes which had originally invaded the peripheral circulation and, in turn, the particular tissue affected, under the influence of chemiotaxis.

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CHAPTER VI

VARIOLA AND VACCINIA IN THE MONKEY

PROFESSOR MICHAEL FOSTER suggested to me some years ago that in view of possible deterioration of lymph stocks, after employment through a long series of generations, it would be well if possible to devise some practical means of investigating the efficiency of the protection afforded by some of those at present in use against variolous infection.

The inoculation of smallpox on the human subject being now a penal offence, I turned my attention to the monkey tribe, on account of their similarity in many respects to man, although assured on high authority that they were not susceptible to either vaccinia or variola. On putting the matter to the test, however, I was agreeably surprised to find that this was not the case, inoculations of vaccine and variolous lymph having each of them given, in my hands, successful results in every instance in which I have tried them on the monkey.

Briefly described, the *modus operandi* has been as follows:—The upper arm, or occasionally the inner surface of the thigh, is shaved on both sides of the

body; the skin is then washed with a solution of some disinfectant, such as corrosive sublimate or carbolic acid, and afterwards with alcohol. Each arm (or thigh) is then scarified in three or four places and the lymph well rubbed in. In the first series of experiments I used ether as an anaesthetic, but I afterwards found this quite unnecessary, as the monkey appears by no means to dislike the scratching process, becoming absolutely quiet as soon as the operation is commenced, and remaining with limbs limp and eyes half closed, as if hypnotised, until its termination, when it at once becomes as lively as ever. In the case alike of variola and of vaccinia the local result of inoculation attains its acme (quâ vesiculation) in the monkey, as in the human being, about the eighth day.

The first signs of reaction appear usually on the third day, by which time, if variolous lymph has been used, there is a distinct though very thin crust over the site of inoculation. In the case of vaccinia the appearance of crust at this date is less marked. By the fifth day vesiculation in both cases has generally commenced, this becoming more obvious up to the eighth day, though even then it is much less marked in variolous cases than in those which have been vaccinated, the difference being recognised with the greatest ease. Later, the vesicle gives place in either instance to a pustule, by which time there is not unfrequently considerable swelling of the skin and subcutaneous tissue and of the nearest lymphatic glands; the pustule gradually dries up, and a scab is formed, which is more

pronounced in the case of vaccination than variolation, and which falls off some time during the third week, if the monkey have not picked it off before.

The chief difference noted between the effects resulting from the local inoculation of these two diseases is, that in the case of variola there is more or less of a crust from the first; that vesiculation is less marked than in vaccination; that about the ninth to the eleventh day a general eruption may appear, which in some instances, as in a monkey which, at the request of Professor Foster, was inoculated at Cambridge, covers the whole surface of the body; and that the final scab at the site of inoculation is not so elevated in the variolated as in the vaccinated animal. In both cases there is usually a rise of body temperature, which is more marked and longer sustained in variola than in vaccinia. After variolation, it was noticed in several cases that the monkey suffered from diarrhœa, that its eyes were suffused, and that it was not as active as usual. A peculiar odour was also noticed, quite distinct from the well-known smell of "monkey." In no instance had the experiment a fatal termination.

Having thus proved to my own satisfaction that monkeys are susceptible not only to vaccination but also to smallpox, I next determined to make trial as to the protection against smallpox afforded in the monkey by previous vaccination, and the protection against vaccination afforded it by variolation, and I went on to compare the effect produced by the use of human and of calf vaccine respectively.

For this purpose humanised lymph was obtained from Birmingham, where, to the best of my belief, the same strain has been continuously carried on by means of arm-to-arm vaccination for the past thirty-eight years. The calf lymph used was obtained from the Government Animal Vaccination Station in Lamb's Conduit Street, while smallpox lymph was supplied to me from the Hospital Ships, from Warrington, and from Manchester. The following experiments will serve as illustration of the system I pursued:—

Experiment 1.—On 13th July 1892 I vaccinated a male rhaesus monkey in four places on the left arm with calf lymph, on points taken at the Animal Vaccine Establishment on the previous day. On 20th July, the eighth day, the appearance presented was undistinguishable from that of a successful case of human vaccination. On 23rd July the vesicles had given place to four large scabs, raised nearly one-eighth of an inch above the surface of the arm, while the surrounding inflammatory zone had nearly disappeared; the axillary glands on the same side were considerably enlarged. A drawing was made of the arm as it appeared on this day by Mr. Lapidge. By 9th August the scabs had all come off, and the monkey was again vaccinated, but this time on the opposite arm in two places, as well as on the inside of the right thigh also in two places, with humanised lymph of ascertained activity. The animal was carefully examined day by day up to 17th August, but no sign of the second vaccination having "taken" was observed.

Experiment 2.—On 19th July a large female rhaesus monkey was inoculated by scarification in eight places (four on each arm) with two tubes of smallpox lymph from the Hospital Ships. The lymph was obtained from a patient aged nineteen, stated to have been vaccinated in infancy, who was suffering from a semi-confluent attack of the disease. The lymph had been sealed up in carefully sterilised tubes. On 23rd July (the fifth day), the right arm of the monkey showed papulation and commencing vesiculation. The axillary glands were slightly enlarged.

On 18th August, a month after inoculation, this monkey was vaccinated with calf lymph of known activity. No result of any kind followed this second operation.

It is, I think, hardly necessary here to enter into details of the numerous experiments which have been performed at intervals of one to five months after the primary inoculation, whether vaccinal or variolous, and I will merely add that in no instance did anything in the nature of a successful result follow the first or subsequent revaccinations. From these experiments it would appear that the protective power of lymph obtained from these three different sources, when inoculated on the monkey, is practically identical in all respects. In discussing the origin of the various lymph stocks at present in use, Messrs. Collins and Picton, in the Minority Report appended to the Final Report of the Royal Commission on Vaccination, make a point of the impossibility of employing at the present day what

used to be known as the "variolous test," as a proof of the efficacy or the reverse of any particular strain of lymph. In view, however, of the results of my experiments with monkeys, this criticism of Messrs. Collins and Picton falls to the ground; since if it is desired to apply the "variolous test" to any given lymph stock, all that is necessary is to vaccinate a monkey with a sample of the lymph in question, and subsequently to inoculate the animal with potent smallpox lymph, after the lapse of such period from the first operation as may be thought desirable.

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CHAPTER VII

ANTITOXIN

In 1891 I placed on record, in a paper presented to the International Congress of Hygiene, certain observations relating to the effects of vaccination with calf lymph containing no living micro-organisms. As was to be expected, vaccination carried out with lymph, previously sterilised by exposure to heat, was not followed by the usual sequence of papule, vesicle, etc., arising at the site of insertion. Nevertheless, the calf which had been operated on by this apparently inert lymph was subsequently found to have been rendered more or less immune against re-vaccination with lymph of normal potency.

My attention was first called to the possibility of thus obtaining temporary protection, at any rate, without production of local result, in the course of experiments carried out with lymph which had been sterilised by heat at a temperature of 60° C. Such lymph not only gave rise to no bacterial colonies in plate cultivations seeded from it, but also, as stated, it produced no local effect whatever when calves were vaccinated with it. I further found that, as in one experiment to which refer-

ence may be made, exposure of the lymph to a temperature as low as 48° C. for an hour had been sufficient to so sterilise the lymph that when inoculated on a calf no apparent result was produced. A week later this calf was inoculated by Dr. Cory directly from another animal, the lymph from which was at the same time used with perfect success on other calves and a number of children. On this particular calf, however, the operation performed by Dr. Cory was altogether without result, thus apparently showing that the use of lymph treated in this manner by exposure to a temperature considerably above that of the body, though itself producing no vesiculation, had rendered the animal insusceptible to the subsequent operation with fresh lymph of normal potency.

On the first occasion on which these results were obtained, I thought that by chance the particular calf might have been the subject of an unusual insusceptibility, but results of later experiments show that this was not the case, since under similar circumstances the result of the second vaccination was invariably negative. In consequence, no doubt remained in my mind that something of the nature of protection had been really afforded by the primary operation in each instance, although nothing in the way of local reaction had been perceptible. A similar course of events has been observed by Chauveau, Klein, and myself, in the conduct of variolation experiments on the calf. These experiments seemed to afford some evidence as to the possibility of obtaining all the desired effects

of vaccination without likelihood of concomitant danger; and I was, therefore, led further to experiment as to the possibility of obtaining similar immunity by the method of subcutaneous injection, which for certain reasons I considered would be preferable to that of multiple superficial incisions on the skin. And as the result of experiment in this direction, by the introduction into the subcutaneous tissue of the back of the monkey of vaccine lymph which had been previously diluted with normal saline solution, in order to have at disposal a quantity of fluid easier of manipulation, I found that some degree of protection was obtainable against subsequent vaccination carried out in the usual manner. In one of the two instances in which I employed this method, a local tumour, possibly an abscess, resulted, probably due to irritation from septic organisms or their products in the lymph.

Chauveau records having pursued a similar method of subcutaneous inoculation of vaccine lymph on bovines and horses; and confirms me in the opinion that by means of a generalised influence over the system, although unaccompanied by production of local result, it is possible to obtain (for a time at least) immunity from subsequent vaccination.

With the hope of throwing some light on this problem, and at the same time making trial of the value of what might possibly afford a new method of protection against smallpox, I devised the following experiment on somewhat similar lines to the well-known work of Tizzoni and Cantani on Tetanus. In a monkey which had

been successfully inoculated with variola five months previously, and which after an interval of three months had again been variolated and also vaccinated without result, the carotid artery was dissected out and tied with antiseptic precautions, after etherisation of the animal. A glass cannula was then inserted into the artery below the ligature and about 30 c.c. of blood received into two test-tubes, each of which contained a few drops of oxalate of potassium solution. The blood, which was thus prevented from coagulating, was then centrifugalised, and rather more than half the total amount of transparent, slightly yellow-tinted plasma was obtained, the red corpuscles remaining in a dense mass at the bottom of the tubes. Of this plasma about 8 c.c. were then carefully inserted into the peritoneal cavity of a second monkey through a minute incision into the linea alba. Two days after the operation both animals appeared perfectly well.

After an interval of fourteen days, on 7th April 1893, the second monkey was vaccinated on the left arm, and also on the inner aspect of the right thigh, with calf lymph on points obtained from the Animal Vaccine Establishment.

I had no opportunity of seeing the animal again till the eighth day, owing to compulsory absence from town; but it was then evident that complete protection, at any rate, had certainly not been afforded by the operation. All the scarified places had "taken," although in each instance the result produced was a somewhat abortive one, as was noticeable on comparing this animal with

another which had been vaccinated on the same day and with the same lymph, and which therefore served as an excellent "control."

Some effect had therefore apparently been produced on the system of the monkey by the procedure adopted, but at the same time it was obviously but slight, even though the amount of plasma injected was considerable, in relation to the size of the animal.

In like manner, the results of experiments in this direction recorded by different observers are all somewhat unsatisfactory as regards the production of a potent antitoxic serum. Thus Kramer and Boyce were unable to produce any appreciable immunity even with large doses of serum separated from the blood of previously vaccinated calves; and, more recently, Beumer and Peiper have arrived at a similar conclusion. On the other hand, Kinyoun, Hlava, and Houl claim successful results; but, so far as can be judged from their publications, such claim would appear to rest on somewhat slender foundation. It may, however, be mentioned that Hlava and Houl record an instance in which injection of their serum to the extent of 0.6 to 1.0 c.c. per kilo of body weight of the experimental calf entirely prevented the action of potent lymph with which this calf was vaccinated four days later by means of superficial incisions on the skin.

The fullest and most recent contribution to this subject is that of Béclère, Chambon, and Ménard. The conclusion at which these authors arrive, as the result of a long series of experiments, is that the serum of

a previously vaccinated heifer, removed after the period of maturation of the vesicles, is capable, on injection into another animal, of conferring a certain immunity against the effects of subsequent vaccination; but the diagrams with which the paper is illustrated show that the antagonism is by no means complete.

In order to obtain the best result, these observers found it desirable to collect the serum at a period of from ten to fifteen days after vaccination of the calf, at which date they found it possessed a maximum degree of potency, as judged by the effects of subsequent vaccination. They state also that the immunising action of the serum obtained from a vaccinated calf is very rapid, since a subcutaneous injection of the serum in sufficient amount, performed on a calf immediately before vaccination of the same animal by means of numerous insertions on the skin, was found capable of modifying the results of this after-vaccination to as great an extent as was apparent in any of their experiments.

When, however, instead of employing for subcutaneous injection the serum from a previously vaccinated calf, they used vaccine lymph in similar fashion, immediately before vaccination of the same animal, the development of the resulting eruption did not appear to be in any way modified. But when vaccination was postponed till the eighth day after subcutaneous injection of vaccine lymph, a somewhat similar result was obtained as in those instances in which, immediately before vaccination, the serum obtained from a previously vaccinated calf had been injected.

The amount of serum which they found it necessary to employ in order to obtain even the incomplete degree of immunity recorded by them, was about 100th the weight of the animal operated on. They further found that the immunising action of their serum was capable of demonstration, to some extent, even when employed for injection at an interval of twenty-four or even forty-eight hours subsequent to vaccination of the animal. Encouraged by the result of their experiments, M. Béclère employed some of the serum obtained from one of their experimental animals for the treatment of a case of smallpox. The amount of serum injected in this case—that of a woman aged twenty-nine years, and weighing 78 kilogrammes—was a litre and a half, given in three successive injections during the course of an hour. The injection of this enormous quantity of serum appeared to cause no local or general injury, and the woman recovered rapidly and perfectly. M. Béclère, indeed, expresses the opinion that the employment of the serum exerted a marked influence in bringing about so successful a result. In his paper he refers to certain other cases in which a similar line of treatment had been attempted, which have been put on record by Dr. Auché of Bordeaux, and Dr. Landmann of Frankfort. These observers, however, employed in the treatment of their cases serum obtained from human beings immunised by a previous attack of smallpox; but the amount of blood withdrawn afforded so small a quantity of serum that it is perhaps not matter of wonder that no appreciable result was found to follow on its use.

About the same time Landmann carried out two further attempts at a similar method of treatment, employing in these instances, however, serum from a previously vaccinated heifer. Dr. Kinyoun and Dr. MacElliot also experimented in this direction in America; but, as in the case of the employment of serum from human beings, the amount of material employed in each instance was again obviously too small to render it likely that any positive result could be expected.

As far as I am aware, M. Béclère and Dr. Kinyoun's cases are the only ones in which successful results are stated to have followed the attempt at treatment of human variola by the antitoxic method. And it would seem probable that no really useful results are likely of accomplishment until we are in possession of some more satisfactory method of immunising the system of the animal from which the serum is derived. Such a consummation can only be expected when further research shall have provided us with reliable methods for the ready cultivation, outside the animal body, of the microbe specific to variola.

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CHAPTER VIII

ANIMAL VACCINATION

By the term "Animal Vaccination," as it is now applied, is meant the employment for the vaccination of the human subject of the virus of cow-pox, as propagated upon a succession of calves or heifers, the original virus having been derived from pocks upon the cow spontaneously affected with the natural disease.

The late Dr. Ballard, in his prize essay on vaccination, gives a most interesting and valuable historical account of the introduction of the practice. In this essay he states that the practice of animal vaccination appears to have originated with M. Negri in Naples, who was seemingly the first to propagate natural cow-pox by successive inoculations upon the heifer. Prior to his time, says Dr. Ballard, cows had been, in various places and by various persons, inoculated with vaccine virus derived from the human subject, the lymph obtained from pocks thus produced being used for human vaccinations. This practice has been termed "retro-vaccination." There appears to be no great difficulty or uncertainty attending retro-vaccination, so long as animals sufficiently young are made its subjects, and the human

lymph is taken under circumstances favourable to its activity. The general result of reinoculation of it upon the human subject has been that the character and activity of the virus thus retransferred remained unaffected by its transmission through the system of the cow. This was the practice first pursued at Naples by Troja in 1805, shortly after the introduction of vaccination into Italy, and also pursued by his successor Galbiati. When, however, M. Negri, in 1842, succeeded to the practice on the death of Galbiati, he soon gave up retro-vaccination, and began to propagate the virus by inoculation from one cow to another, that first propagated being of human origin. Subsequently, on the occasion of an outbreak of cow-pox in Calabria, the use of this virus was abandoned, and M. Negri propagated thenceforth that obtained from the natural cow-pox. It is said that on two occasions he thus availed himself of natural cow-pox matter obtained in Italy, but that on a third occasion, in 1858, on which he renewed his supply, it was derived from London.

A good deal of doubt has been thrown upon the origin of this third renewal of virus, and it has been questioned whether it was virus from natural cow-pox at all. However, it appears that it is this virus which M. Negri propagated subsequently to 1858. In 1864 M. Lanoix, a young French physician, visited Naples in order to study the practice of animal vaccination as pursued there. On his return, he brought back with him to Paris a calf inoculated with the virus then in process of transmission by M. Negri, and in concert

with M. Chambon set up a private establishment for the propagation of the virus from calf to calf, and for the supply of animal vaccine in Paris.

In his report on vaccination in France during 1864, M. Depaul in the name of the Académie de Médecine gave an account of the observations made in Paris on this subject, and in February 1866, the French Government placed a sum of 6000 francs at the disposal of the Académie for the purpose of experiment in the matter. A Commission was appointed, the report of which was drawn up by M. Depaul. This report was favourable to the practice of animal vaccination. The Commission, however, were much divided in opinion, and the Académie did not then recommend that the practice should be adopted. At first, the virus used by the Commission was that in use by MM. Lanoix and Chambon, and which they had previously obtained from Naples. But after four transmissions of this lymph had been made, a new source of virus from natural cow-pox was discovered at Beaugency (Loiret), and then the use of the Neapolitan strain was abandoned and this new and undoubted cow-pox virus alone employed. MM. Lanoix and Chambon also adopted the use of this strain to the exclusion of that they had before employed, and having, in the autumn of the same year (1866), met with another case of natural cow-pox at St. Mandé, near Paris, they introduced this lymph also into their practice of animal vaccination. They saw no advantage in keeping these two lymphs distinct, and the strain which they have employed from 1866 onwards is a mixture of

the two natural sources discovered at Beaugency and at St. Mandé.

Whatever the origin of the Neapolitan virus may have been, therefore, there can be no question that the inoculations made by the Commission (after the first four), and those afterwards made in Paris by MM. Lanoix and Chambon, were transmissions of the virus of a cow-pox of spontaneous origin.

From Paris the practice of animal vaccination extended in many directions. Thus in February 1865, it was introduced into Brussels by Dr. Warlomont, whose first supply of lymph was obtained from M. Lanoix (Neapolitan source). At a later period, he obtained from Paris virus in course of propagation from the Beaugency source, and, still later on, he introduced a third source obtained in July 1868 at Esneux (Liège).

In September 1866, at the request of the Minister of the Interior, the Royal Academy of Belgium referred the whole question to a commission consisting of MM. Bellefroid, Thieruesse, and Marinas, who reported favourably upon it, and, in consequence, in July 1868, an "Institut Vaccinal de l'État" was established at Brussels, under the direction of Dr. Warlomont, for maintaining the practice of animal vaccination, and distributing gratuitously lymph obtained from the animals, or lymph taken from children who had been vaccinated direct from the animals, according to the preference of the vaccinators or other practitioners demanding it.

The practice was introduced into Berlin by Dr.

Pissin in June 1865, and the establishment which he founded is still carried on as a private .speculation. Animal vaccination appears to have been introduced into Vienna in a similar manner about the same time, and many of the great capitals of Europe rapidly followed suit. Thus, early in 1868, the Society for the Promotion of Cow-pox Inoculation at Rotterdam determined to open a station for the practice of animal vaccination, and they commenced operations in April of that year, distributing lymph throughout Holland and its colonies. A similar course was adopted in the following year by the corresponding society of Amsterdam.

Switzerland also owes the comparatively recent introduction of animal vaccination into that country to private enterprise; what is now known as the Institut Vaccinal Suisse having been founded in 1882 by M. Charles Haccius, the present Director of the establishment. Originally a private venture, the Institute, which is situated at Lancy on the outskirts of Geneva, is now recognised by the various cantonal governments, M. Haccius, in consideration of an annual subvention, supplying to public vaccinators throughout Switzerland, free of cost, all the lymph required by them in the performance of their duties. This establishment I have recently (1897) had the opportunity of visiting, in the course of a tour of inspection, at the instance of the Government, of certain of the chief calf-vaccine establishments on the Continent of Europe.

At Paris, as is the case with the establishment of M. Haccius at Lancy, the Institut de Vaccine Animale

is practically a private concern, although the municipality of the city of Paris contract with the Directors to carry out all such public vaccinations, within their jurisdiction, as may be necessary. This institution, which is now carried on by M. Chambon and Dr. St. Yves Ménard, was founded, as already mentioned, by M. Lanoix. Originally situated in the Rue Massillon, it was subsequently transferred to the Rue Ballu, where what was formerly a dwelling-house has been adapted to its present purpose.

In Germany all the establishments visited by Sir Richard Thorne and myself, at Berlin, Dresden, and Cologne respectively, are under Government control. Of these, that at Cologne is the most recent of its kind, and its buildings with their fittings are of the most modern description. This Institution, of which Dr. Vanselow is the Director, was erected in 1889 for the production of animal lymph, to meet the needs of the Rhine province and Hohenzollern districts. The building adjoins the central meat market, a matter of convenience for the obtaining and subsequent disposal of calves. On the ground floor of the building are the collecting room, calf stable, slaughter room, director's and clerk's rooms, corridor, and offices, while on the first floor is a bacteriological laboratory, fitted up with all needful apparatus, which is reached from the collecting room by an iron spiral staircase.

At Brussels, the propagation, storage, and distribution of calf lymph is carried out at the École Véterinaire, under the supervision of Professor Dégive,

the Director of the establishment. The accommodation provided in the present building is regarded as very insufficient for the purpose, and accordingly, as Professor Dégive informed us, a new vaccinal institute is about to be erected, in the construction of which all modern scientific requirements will be met. The private establishment, originally founded by Dr. Warlomont, and which is now carried on by his son-in-law, an officer in the Belgian army, we had not the opportunity of visiting.

Although the practice of animal vaccination is now practically universal throughout Continental Europe, in England up to the present time it has been employed to a very limited extent. At the Government Animal Vaccine Establishment at Lamb's Conduit Street, which was founded in 1881, as the result of investigations by the late Sir George (then Dr.) Buchanan and Dr. Cory, direct vaccinations of children, from calf to arm, have been carried out from that date onwards to the present time.

From the calves vaccinated at Lamb's Conduit Street, lymph has also been supplied to public vaccinators in quantity sufficient to enable them to start their periodical vaccinations, and provision has also been made for supplying a comparatively small amount of lymph to private practitioners in addition. The lymph thus sent out has for the most part been preserved by drying on ivory "points," although a certain small proportion has been stored in the liquid form in capillary glass tubes.

Until quite recently, however, all public vaccinations in this country, with the few exceptions just mentioned, were required to be performed by direct transmission of the virus from arm to arm, stational attendances at fixed periods having been arranged for in order to render feasible such method of operating; weekly attendance having been provided in large towns, while quarterly or even half-yearly periods sufficed for the required purpose in scattered country districts.

As the result, however, of the findings of the Royal Commission on Vaccination, legislative measures have now been introduced by which arm-to-arm vaccination will be entirely superseded, in public work at any rate, by animal vaccination; and the adoption of the use of preserved glycerinated calf lymph having been determined on, the necessity for stational attendance will disappear, except in so far as may appear desirable for purposes of control or in order to provide facilities for teaching.

The principal grounds on which the practice of animal vaccination has been advocated are:

1. The opinion, widely though not universally held, that the results of vaccination from arm to arm are not such as they used to be in the earlier years of the practice of vaccination; in fact that by repeated human transmission the virus has become weakened, and that the pocks produced by the introduction of lymph which has passed through a large number of human beings are not so fine or so perfect as those which result from the use of animal lymph. Jenner himself indeed appears to have

been under the apprehension that lymph-stocks would be liable to degenerate as the result of long-continued transmission through the human subject.

In the present state of our knowledge, however, such enfeeblement of the specific virus can hardly be regarded as probable, except under conditions that may be obviated by reasonable skill and care on the part of the operator.

Jenner early discovered that vaccine lymph only exhibited its full degree of activity when taken at the stage of maturation of the vesicle, and before its contents became at all purulent. If this precaution be observed, together with strict cleanliness in removal and insertion of the lymph, experience has shown that no appreciable degeneration can be demonstrated.

2. The opinion that certain other human diseases may be propagated together with vaccinia, when vaccinations are performed from arm to arm. Galbiati, who was one of the first, if not the first, to vaccinate with bovine lymph, adduced this belief as his reason for practising vaccination from the cow; arguing that on vaccinating the cow from the human subject vaccinia alone would be communicated to her, any other morbid germs which might be introduced along with it remaining without effect. He considered apparently that in practising retro-vaccination according to the method which he devised, he, as it were, filtered human vaccine material from any possible contamination.

As the result of all the evidence brought before them during the lengthy period of seven years, the

English Royal Commission have reported that the risks incurred from arm-to-arm vaccination "are undoubtedly real and not inconsiderable in gross amount," although "when considered in relation to the extent of vaccination work done they are insignificant." And they add that "there is reason further to believe that they are diminishing under the better precautions of the present day, and with the addition of the further precautions which experience suggests will do so still more in the future."

It is indeed the fact that cases of syphilis have been occasionally conveyed from one human being to another as the direct result of vaccination; and although the number of cases that can be authenticated is extraordinarily small in number, there is advantage in the adoption of a method which, while equally efficacious, entirely obviates the possibility of so lamentable an occurrence.

Erysipelas and tuberculosis are also two of the not inconsiderable number of diseases with the spread of which vaccination has been credited. No doubt the greater proportion of cases of erysipelas following on the process are of what has been termed "late" origin. These are due to infection of the vaccination wound at or about the time when it has been customary to open the vesicle. The general use of animal vaccine, by obviating the necessity for opening the vesicle, may be expected to be followed by a diminution of cases originating in the manner suggested, though accidental injury to the arm, or the use of improper dressings, will

doubtless prevent the complete disappearance of erysipelas or other septic diseases. Invaccinated erysipelas however, we may anticipate, will shortly become in great measure, at any rate, a thing of the past; and, fortunately, the possibility even of the invaccination of tubercle may be eliminated as the result of the treatment of calf vaccine with glycerine as now officially adopted by the Government, after a method which I first advocated some years ago, and to which I at once proceed more particularly to refer.

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The Final Report of the Royal Commission on Vaccination may also be consulted with advantage.

CHAPTER IX

GLYCERINATED LYMPH

In a paper presented to the International Congress of Hygiene held in London in 1891, and subsequently published in the Transactions of that Congress, I called attention to a special method for the bacteriological purification and preservation of vaccine lymph. This method consisted in the intimate admixture of a given amount of lymph, or rather vesicle pulp, with a sterilised 50 per cent solution of chemically pure glycerine in distilled water, and in subsequent storage of the resultant emulsion, in sealed capillary tubes, for several weeks.

For some years antecedent to 1891, I had been engaged in investigating the nature and mode of action of the specific virus contained in vaccine lymph, chiefly from the bacteriological point of view. Early in the course of my experiments it struck me that the exuberant growth commonly manifested by what were so evidently "extraneous" organisms, might be very possibly interfering with, if not superseding, the more important and essential organism that I was seeking. I therefore turned my attention to the discovery, if possible, of some means of so treating vaccine lymph as to inhibit

the multiplication in it of all "extraneous" organisms, and eventually to destroy them altogether, without at the same time injuring its potency for vaccination. These bacteria evidently find in the lymph, especially when removed from the body, a suitable medium for their subsequent multiplication, while at the same time it would appear that growth and multiplication of them has as result the gradual inhibition of the specific effect proper to the vaccine virus itself. Upon all grounds, then, the obvious indication for my guidance was not only to prevent such multiplication of "extraneous" organisms subsequent to storage in the usual manner, but, if possible, to remove them altogether as soon as the lymph was taken from the living subject, without injury to the actual contagium of vaccinia.

Without detailing the various experiments adopted with this object in view, it is necessary here to state briefly the lines on which the work was carried out. In the first place, trial was made of the method of exposure of lymph for definite periods to a temperature considerably above blood-heat, which had, in the hands of Kitasato, met with conspicuous success in the isolation of the bacillus of tetanus. Proceeding in this manner, and in every experiment observing the precaution of making control cultures, I presently arrived at a temperature, exposure to which is apparently incompatible with the continued existence of those micro-organisms which can ordinarily be grown when vaccine lymph is inoculated into nutrient jelly. The temperature in question ranged between 38° C. and 42° C., but the

method did not in practice prove advantageous. Thus, if plate cultures were made of lymph after exposure for an hour at the lower register, a few points of growth occasionally after the lapse of a day or so made their appearance; and, on the other hand, the higher temperature, though it inhibited all extraneous microbes, appeared sometimes to exert an injurious effect on the lymph, in so far as regards the normal vesiculation which should result from its inoculation in the living animal. Some method of readier application, and requiring less delicate manipulation, was therefore obviously desirable. This I at length found in the addition to the lymph, or rather to the vesicular pulp, obtained from a vaccinated calf, of a sterilised 50 per cent solution of glycerine in distilled water.

Admixture of glycerine and vaccine lymph is, of course, no new device. Indeed, my attention has recently been directed to a letter by the late Mr. R. Cheyne, which appeared in the *Medical Times* as long ago as March 1850, in which he advocates the superiority of lymph which has been kept in the fluid state by addition of "the least portion" of glycerine over that which had been dried on points. As Mr. Cheyne's work appears to have been overlooked until quite recently, it may be of interest to set out in his own words his mode of procedure. "My method," he says, "is to take all the lymph I can get from the eighth day vesicle on a glass stopper (elongated in a narrow tongue-shaped form for about an inch below the neck of the small bottle into which it fits), and having accumulated

it on both surfaces of the stopper near its end, I mix it well with the point of the probe with the glycerine held by the latter. Then after the stopper is put into the bottle (which is always to be kept standing up), the lymph, then assisted by gravity, will collect itself into a distinct drop on each surface of the stopper to be ready for use, as I know by experience, during a very long period, and in any number of cases, that would not exhaust the stock." In a further letter Mr. Cheyne acknowledges his indebtedness to the late Mr. J. Startin's advocacy of the therapeutic applications of glycerine, by which he learnt of the suitability of this substance for the object he had in view, and sets out, practically in the wording of Mr. Startin's paper, the distinctive properties of this substance. He goes on to say, "I mixed glycerine with vaccine lymph in the way detailed, . . . with the satisfactory result, better than any theory, of discovering that, in addition to its known property of preventing fermentation and mouldiness in vegetable substances, it had also that of keeping vaccine lymph, an animal product, undecomposed in a fluid state for months." Three years later (in 1853), Mr. Cheyne showed to the Presidents of the Royal Colleges of Physicians and Surgeons a child whom he had successfully vaccinated with lymph which he had previously kept for six months after treatment with glycerine.

It was left for Müller of Berlin, in 1869, to demonstrate the further point that the quantity of material available could be increased by the addition of glycerine

without injuriously affecting the potency of the mixture. He showed that vaccine lymph might be diluted with three times its bulk of glycerine, and still retain its properties unimpaired, a fact which has been taken advantage of at many of the continental vaccine stations, and by more than one purveyor of lymph. Cursch-mann, writing on the subject of smallpox in Zeimsenn's *Encyclopaedia*, refers to this method as follows :—" Müller has the great credit of having discovered the fact that by mixing vaccine matter with glycerine in certain pro-portions, the activity of the former is not diminished, so that we have here a means of *increasing the volume of the lymph* when the quantity is small or when there is an unusual demand for it. . . . The lymph and glycerine mixture *appears to keep as well as the unmixed lymph.*" [1] But from this statement it is quite obvious that the sole object of employing glycerine in the manner described was to increase the amount of material available for vaccination.

Also with this end in view, glycerine was used by Dr. Stephen Mackenzie, at the London Hospital, in the epidemic of 1870-71, the mixture of lymph and glycerine being made up immediately before it was required for a large series of revaccinations. Similar means for increasing their amount of available lymph have been frequently employed by public vaccinators (among the earliest, my present colleague, Dr. Bruce Low) and others in times of stress.

[1] The italics are my own.—S. M. C.

In 1882 Dr. Warlomont patented, in England, a method of admixture of glycerine with vaccine lymph, but no mention of the use of glycerine in this connection was made in the advertisements of his agents until within the last few months. Until recently, moreover, there would seem to have existed no appreciation of the inhibitory action exerted by the glycerine in bringing about bacteriological purification of the lymph, when the mixture is stored for some time prior to use, under conditions preventing access of air and light.

When, however, a glycerine emulsion is properly prepared after the method I have advocated, it is found that the growth of extraneous aërobic bacteria is at once greatly inhibited, while after a longer or shorter period they are practically all killed out. This effect is best demonstrated by making a series of plate cultivations from tubes of glycerinated lymph, at gradually increasing intervals of time, a control plate being established in each instance from a specimen of the lymph material prior to the admixture of the glycerine. These observations of mine, since their publication in 1891, have received ample corroboration from a number of observers in various parts of the world, as follows:—

In 1892, a paper dealing with this question was published by MM. Chambon and St. Yves Ménard, in which they relate their experience of the use of glycerinated calf lymph when kept for a considerable period in capillary glass tubes (previously sterilised) closed by the blow-pipe. Not only were the results they obtained with originally active lymph highly satis-

factory, but lymph which, in its fresh state, had given mediocre results, produced, after fifteen days' admixture with glycerine, a passable vesicle, and, after forty, fifty, or sixty days, a typical one. The improvement in the activity of such lymph seemed to them to be due to the gradual extinction of extraneous microbes under the combined influence of glycerine and length and method of storage. Professor Straus, who made plate cultures with their glycerinated lymph, found that, when fresh, it gave rise to numerous colonies of various microbes, especially staphylococcus pyogenes aureus and staphylococcus albus, but that when it had been stored from fifty to sixty days, plate-cultures therefrom remained absolutely sterile as regards these extraneous microbes. Samples tested at intervals between these two extremes presented fewer and fewer microbes as they became older. These experiments were repeated many times, and invariably with similar results. This evidence, so entirely corroborative of my own work, is the more important as it appears certain, from a perusal of their original paper, that the authors were ignorant that similar results had been previously arrived at, and that these had been published nearly twelve months before the appearance of their article.

The value of glycerine in this connection is also strongly advocated by Leoni in a paper read before the Medical Congress held at Rome in April 1894, and afterwards published in the *Revue d'Hygiène*. He finds that vaccine lymph, as freshly collected, is apt to contain large numbers of micro-organisms, some of which, he

says, are capable of exerting pathogenic properties when inoculated into the system along with the true vaccine virus. And he states that these microbes disappear completely from, or that at least their number is vastly decreased in, vaccine which, having been prepared with glycerine, is afterwards preserved for a period of from one to four months before use. His conclusions may perhaps be best given in his own words:—"Le vaccin récemment recueilli est un vaccin contaminé." "Les agents de la contamination s'épuisent dans le vaccin conservé pendant quelque temps dans la glycérine." "Le vaccin conservé dans la glycérine pendant 1 à 4 mois après la récolte, représente le type du vaccin *pur*, d'une *virulence uniquement spécifique.*" "C'est de cette qualité de vaccin que l'hygiéniste doit aujourd'hui tenir compte dans la prophylaxie de la variole."

Dr. Klein also bears witness to the power exerted by glycerine in freeing vaccine lymph from bacteria. In speaking of the organism specific to vaccinia as being probably a spore-bearing bacillus, he says: ". . . it is established that the active principle of vaccine is preserved in glycerine, although, as is also known, glycerine acting for long times is a germicide for cocci and sporeless bacilli."

In 1896, a Commission under the Presidency of Dr. Schmidtmann, and including Dr. Koch, Dr. Pfeiffer, and Dr. Frosch, together with the Directors of the Vaccine Institutes of Berlin, Cologne, and Stettin, was appointed by the German Government to inquire and report as to the best methods for the collection, preser-

vation, storage, distribution, and use of vaccine lymph. In their report, which has been recently published, the Commission arrive at the conclusion, among others, that fresh lymph contains numerous microbes, the number of which on the addition of glycerine diminishes as the age of the mixture increases. To determine to what extent glycerine is efficacious in destroying the vitality of various definitely *pathogenic* microbes, numerous streptococci and diphtheria bacilli were mixed with specimens of lymph. As a result, the streptococci were killed in eleven days and the diphtheria bacilli in twenty days.

Attempts by these experimenters, as also by Kitasato in Japan, to make other chemical agents serve for rendering vaccine lymph free from bacteria led to no results of value, as although the lymph could thus be rendered free from extraneous bacteria, it was found to be inefficacious as vaccine. Their next procedure was to determine the amount of glycerine that could be added to lymph, so as to exert a powerful action in purifying it from extraneous microbes, without in any way interfering with its specific action when employed for the vaccination of children or calves, and they came to the conclusion that the mixture of glycerine with distilled water could be employed to the extent of from fifteen to twenty times the weight of vesicle pulp collected, without interfering with the value of the material for the purpose of vaccination.

The fact that the growth of the tubercle bacillus is encouraged in a nutritive medium containing about

6 per cent of glycerine, in no way militates against our experience as to the germicidal value of this substance, when used in a strength of from 40 to 50 per cent. That certain drugs, when introduced in small quantities into the human economy, produce effects vastly different to those resulting from the introduction of the same drug in large quantities, is a fact familiar to every practitioner, and it is also well known to bacteriologists that micro-organisms are profoundly affected by variations, in one or another direction, in the chemical composition of the particular medium in which they are placed.

By consent, therefore, of numerous observers fully qualified to judge of the matter, we have in glycerinated calf lymph, properly prepared, a vaccine material which, while even more efficient as vaccine than the original lymph, can be produced practically free from the extraneous organisms which, at one time or another, have been isolated from fresh or stored lymph by the method of plate cultivation. Of not least importance is the fact that, as shown by Dr. Blaxall and myself, vaccine lymph may by this method of preparation with glycerine be rendered free also from pathogenic bacteria, such as those of tubercle and erysipelas, even when these have previously been added in considerable quantity for experimental purposes.

At a meeting of the British Association held at Liverpool in 1896, Dr. Blaxall and I communicated a report on the "Influence of Glycerine upon the growth of Bacteria."

In view of the publication of the Report of the Royal Commission on Vaccination, and of the reference to my previous work contained therein, it had appeared to us desirable to investigate more accurately the action of glycerine on various definite micro-organisms of a pathogenic or non-pathogenic nature respectively.

For the purposes of this investigation quantities of glycerine were added to tubes of beef-peptone-broth, which were subsequently inoculated with equal quantities of pure cultivations, and incubated at blood-heat and at the room temperature respectively. Control inoculations in ordinary beef-broth were also invariably employed. Subsequently inoculations were made from the broth tubes on to solid media at varying intervals of time, in order to see whether the particular microbe still remained capable of growth or not. In all, some hundreds of inoculations were made, and the paper included a table in which were given the maximum limits of resistance attained in the different series.

Practically, the results of each similar series were found to agree very closely.

The micro-organisms employed for the inoculations comprised Staphylococcus pyogenes aureus, S. pyogenes albus, Streptococcus pyogenes, Bacillus pyocyaneus, B. subtilis, B. coli communis, B. diphtheriæ, and B. tuberculosis. Smallpox and vaccine material in the form of "crusts" and lymph was also employed.

Results.—1. No visible development of the micro-organisms employed took place in the presence of more than 30 per cent of glycerine.

2. None of the micro-organisms experimented with could be recovered after exposure for a month to the action of from 30 to 40 per cent glycerine, with the exception of B. coli communis and B. subtilis when kept in the cold.

3. B. coli communis, unlike B. typhosus, resists the action of 50 per cent glycerine in the cold for a considerable period, a fact likely to prove of value as an addition to our present methods of differentiating these microbes one from another.

4. The samples of smallpox and vaccine material, whether as "crusts" or lymph, were sterilised completely, so far as extraneous microbes are concerned, in a week, by the presence of glycerine to the extent of about 40 per cent in the broth-tubes. This short period of resistance is, doubtless, in part, to be explained by the fact that the smallpox crusts used in these experiments had been obtained several months beforehand. Presumably therefore the number of microbes, which had been able to survive for so long a period the process of drying, would be much less than might be expected to be present in "crusts" recently obtained.

In this paper also, we published an account of some experiments relating to the action of glycerine on certain yeasts; under this heading being included, for reasons of convenience, both Saccharomyces and Torulæ.

The results show that these organisms vary greatly in their resistance to glycerine, those not unfrequently found in vaccine material being easily killed out, whereas the common pink yeast (Rosa Hefe) resists the action

of 50 per cent glycerine for an almost indefinite period.

Since the date of the paper referred to, we have instituted a further series of experiments upon the tubercle bacillus, with the object, now, of determining whether this organism can survive, and remain capable of further development, after a sojourn for varying periods in glycerinated vaccine lymph. For this purpose vaccine material was rubbed up in the usual way with a sterilised mixture of 50 per cent glycerine and water. The greater part of the resulting emulsion (containing glycerine to the extent of 42 per cent) was at once stored in small, 3-gramme, test-tubes. To the residue, amounting to about 4 c.c., was added a large quantity of growth from a culture of tubercle bacilli recently isolated and of full activity. This growth was thoroughly mixed with the emulsion, and the whole was poured into two 1-gramme test-tubes which were corked and placed, with the rest of the tubed emulsion, in a cupboard in which our lymph is stored. This cupboard is kept at about 15° C. by means of ice. At the same time control inoculations were made from the tubercle culture in tubes of 6 per cent glycerine agar-agar, and in tubes of 6 per cent glycerine-peptone-beef-broth (Roux's Media). These latter were then incubated at 37° C.

At the end of a month the glycerinated vaccine emulsion was demonstrated, by means of plate-cultivations, to be free from extraneous organisms; and, similarly, plates poured from the small tubes containing the tubercle bacilli also showed no growth.

Inoculations were made from these small tubes on to tubes of 6 per cent glycerine agar-agar, and on to tubes of solidified blood serum. These were incubated at 37° C. After a month's incubation, no growth resulted from any of these inoculations. Lest traces of glycerine carried over with the inoculation needle should have retarded or prevented the growth of the tubercle bacillus, some of the emulsion was freely mixed with sterile beef-broth, and from this also numerous inoculations were made and incubated at 37° C. These again, after the lapse of a month, failed to show any sign of growth.

The control tubes inoculated with the growth of tubercle employed in the whole series of experiments and incubated at 37° C. all exhibited a copious growth in a month, and sub-cultures from them were all in turn successful.

As the result of our different series of experiments with the tubercle bacillus, then, it has been found impossible to recover this micro-organism after exposure for a month to the action of glycerine present to the extent of about 40 per cent, either in sterile beef-broth or in fresh vaccine material.

Valuable corroboration of our work in this direction is afforded by the results, as yet unpublished, of certain experiments carried out by Dr. Klein, which demonstrate the germicidal influence of glycerine on the tubercle bacillus. These I am now enabled by the courtesy of Dr. Klein to place on record.

"10*th February* 1897.—From a typical tubercle

culture put a good deal of growth into 50 per cent glycerine broth. After shaking, made two inoculations on slanting surface of glycerine agar; sealed and incubated at 37° C. . . .

"24*th February*.—Good growth of tubercle bacilli in both tubes. . . .

"9*th April*.—From glycerine-broth tubercle mixture of 10th February, inoculated two glycerine-agar slanting surfaces; sealed and put in incubator at 37° C. . . .

"23*rd April*.—No growth in any tube. . . .

"1*st May*.—No growth in any tube. . . .

"5*th June*.—No growth in any tube. . . ."

On 9th April 1897 Dr. Klein "injected two guinea-pigs subcutaneously with a good dose of the glycerine-broth tubercle mixture. There was no effect produced either locally or generally."

Dr. Blaxall and I, feeling that cultivation experiments alone could not be held to be absolutely conclusive, have also carried out a series of injection experiments on guinea-pigs. The material we employed consisted of glycerinated vaccine lymph emulsion to which at the time of its preparation, at least a month before testing it by means of subcutaneous injection, virulent tubercle bacilli had been added in large quantities. For the purpose of these experiments two tubes, each containing three grammes of glycerinated calf lymph, were inoculated, immediately after preparation of the lymph, with three large platinum wire loopfuls, to each tube, of tubercle bacilli from a culture of proved activity. After securing

as thorough admixture as possible of the culture material with the lymph emulsion, cover-glass specimens were made from the mixture, and stained in appropriate fashion. All of these preparations on subsequent examination by the microscope showed large numbers of tubercle bacilli in every field. Both tubes were then set aside for a month, at the end of which the tubercle-containing vaccine lymph emulsion was employed for subcutaneous injection of guinea-pigs, other animals being at the same time injected with equivalent portions of the original growth of tubercle in order to serve as controls. These latter in due course developed tuberculosis locally, which subsequently became generalised, while, on the other hand, the animals which had been inoculated with the tubercle-containing vaccine lymph emulsion showed no result either locally or generally.

The question of quality of vaccine lymph naturally engaged the attention of the English Royal Commission on Vaccination at the time they were taking the evidence of witnesses, and among the recommendations contained in their final report is one to the effect that no persons should be required to submit to vaccination by means of any other lymph than that derived from the calf. The reason for this recommendation appears to be avoidance of even remote risk of inoculation, in the process of vaccination, of infections other than cow-pox—such, for instance, as those of syphilis and leprosy (sect. 433)—as also the desirability of minimising the opportunity for infection by erysipelas by obviating the necessity for opening vaccine vesicles involved in arm-

to-arm vaccination (sect. 447). The succeeding section of the report, which deals specially with the subject now being discussed, may be set out in full. It is as follows :—

448. We think that safety would be increased by preserving the lymph in tubes instead of on "dry points." There is some difference of opinion on this matter among those with whose opinions we have been furnished. On the whole, however, we think the weight of experience as well as reason is in the direction we have indicated.

In connection with this subject, our attention has been drawn to the experiments recently made by Dr. Copeman as to the effect of the storage of vaccine lymph in glycerine. The conclusions at which he arrives are that the addition of glycerine, whilst it leaves the efficacy of the lymph undiminished, or even increases it, tends to destroy other organisms. If it be the fact that the efficacy of the lymph remains unimpaired, its storage in glycerine would largely diminish the difficulties connected with the use of calf lymph which are inseparable from calf-to-arm vaccination. The investigation has not reached a point at which it is possible to pronounce with certainty whether the anticipated results would be obtained. And it was at one time suggested that the

> introduction of glycerine was likely to be mischievous. The question is one a further investigation of which is obviously desirable. If lymph is to be preserved in glycerine, due care would be requisite to ensure its purity and the absence of contamination in its introduction. We think that, whether mixed with glycerine or not, each tube should contain only sufficient lymph for the vaccination of one person. (August 1896.)

In this section (448) of the Royal Commissioners' Report, a sentence occurs to which some special reference is desirable. This is as follows:—"And it was at one time suggested that the introduction of glycerine was likely to be mischievous." The reference made is to an outbreak of disease having the clinical characters of impetigo, which occurred in the summer of 1885, in villages situated on the island of Rügen, in the Baltic, after the vaccination of seventy-nine children in the latter part of June of that year.

This outbreak forms the subject of a paper by the late Sir George Buchanan in the *Transactions of the Epidemiological Society* for 1885-86. He states that for the vaccination of these seventy-nine children only two tubes of lymph (of human origin) were available, and consequently their contents were further mixed with glycerine (glycerinum purissimum) before use. Thymol, to the extent of one-third, had also been mixed with the lymph previous to its having been sent out from the Government Establishment at Stettin. Sir George

Buchanan states that the principal misgivings of the Commission appointed to inquire into the outbreak had concern with the glycerine, but adds that several points in the circumstantial evidence, however, suggest that the Stettin mixture, *before glycerine had been added*, may have been responsible for some irritation. He further says, however, "I do not know of any experience in England of inoculation with thymol or thymol and glycerine, but I have heard of dilutions of lymph with glycerine, always from people complaining of the lymph."

In giving evidence before the Royal Commission on Vaccination in 1893, I was asked whether this statement did not condemn my suggested use of glycerine in connection with lymph supplies. I pointed out, however, that, in the first place, dilution of lymph with an indefinite quantity of "glycerine" of unknown composition just before employing the mixture for vaccination, was a very different matter from storing lymph with chemically pure glycerine in definite amount and of definite strength, the whole being protected from light and air for a length of time before use, and that, in addition, what at the time of the outbreak in question may have been termed glycerinum purissimum, would in all probability at the present day be considered by no means deserving of that term. This latter statement I made on the authority of Messrs. Price, the chief manufacturers of glycerine in this country, whose manager, at the request of the directors, was good enough to put at my disposal his extensive practical knowledge of the details and the literature of this

subject. The information thus obtained has been published in the appendix to the last volume of evidence, recently issued by the Royal Commission on Vaccination.

The glycerine which has been employed in my investigations is that manufactured in this country by Messrs. Price, but in Germany, where this method of purification and preservation is now carried out in all the Government Establishments for the supply of vaccine lymph, a glycerine made by Sarg of Vienna is used. This is said by Dr. Schultz, the Director of the Berlin Establishment, to be of a less "drying" nature than English glycerine.

In order to obtain an authoritative opinion as to the degree of chemical purity obtained in the manufacture of glycerine at the present time, and also an accurate estimate of the difference, if any, in the nature of the English and Austrian brands, I requested Dr. Wilson Hake to analyse samples of glycerine manufactured by Messrs. Price and by Sarg respectively, and also samples of a new English brand which had just been placed on the market by Messrs. Lever Brothers. The result of the chemical examination of these samples, which Dr. Hake was kind enough to make for me, proves incontestably that all three samples exhibit a high degree of purity, most markedly so in the case of Messrs. Price's manufacture. In all there is complete absence of metallic contamination of any kind, while the amount of organic impurity is insignificant. The main difference observed is the slightly greater degree

of concentration obtained in the English products as opposed to that of Austrian manufacture.

In accordance with the suggestion contained in section 448 of the Report of the Royal Commission, the Local Government Board instructed me to make arrangements with Dr. Blaxall, the Lecturer on Bacteriology at Westminster Hospital, and now Bacteriologist to the Government Glycerinated Lymph Laboratories, to carry out a further series of experiments on the lines indicated by me in the evidence which had been tendered to the Commission, with the object of elucidating more fully the exact value of my glycerine method for the purification and preservation of vaccine lymph. The results of this series of experiments are set out in the Report of the Medical Officer to the Local Government Board for the year 1895-96, published in the autumn of 1897.

Although devoting our attention mainly to the results which ensued on the employment of glycerine, it was thought desirable that investigation should be made concurrently as to the possible value for the preservation and purification of vaccine material, of lanoline and vaseline, substances which within quite recent years have been introduced by two officers of the Indian Medical Service, Surgeon Lieut.-Colonel King, and Surgeon-Major Bamber respectively, as being superior in their opinion to glycerine as agencies for the preservation of vaccine.[1] Neither of these observers, how-

[1] The use of vaseline as an excipient for vaccine material had apparently been advocated by Professor Layet of Lyons, some years previous to Surgeon-Major Bamber's first communication on the subject.

ever, has as yet brought forward any bacteriological evidence as to a possible germicidal action of the substance they advocate, as will be seen on reference to their original papers. And Dr. Blaxall has shown that from this point of view, at any rate, neither lanoline nor vaseline can be regarded as possessing any value whatever. Indeed, it would appear that the extraneous organisms originally present in the lymph, with which these substances are admixed, so far from exhibiting as time goes on any diminution in numbers, tend, on the contrary, to become largely increased, as shown by the test of plate-cultivation.

Lanoline and vaseline are thought by their advocates to possess superiority over glycerine mainly for the following reason. These officers note that glycerine emulsion tends to become mouldy after a short period, whereas this they do not find to be the case when either lanoline or vaseline is employed. That glycerinated lymph should have suffered deterioration in the way they indicate suggests either that it was not properly prepared, or that it was left exposed to the air. That the occurrence of a similar accident is not an impossibility with lanoline and vaseline preparations; has been demonstrated by Dr. Blaxall's recent experience of their use.

As examples of the results obtained, I set out in full the record of two experiments in which *the action of glycerine, vaseline, and lanoline respectively on vaccine material is compared.* I append also the record of one experiment in which glycerine only was employed, but in which the glycerinated vaccine pulp was employed by

Dr. Cory for the vaccination of a considerable number of children at the Government Vaccine Establishment, Lamb's Conduit Street.

The glycerine used was Price's pure glycerine, having a specific gravity of 1200. It was thoroughly mixed with sterilised distilled water to 50 per cent, being weighed, and the resulting mixture was again sterilised.

The vaseline used was pure vaseline, which, when bacteriologically tested, was found to be sterile. The lanoline used was pure anhydrous lanoline, and this also had been found to be sterile.

The vaccine material was collected from calves which had been vaccinated in the ordinary course at the Animal Vaccine Establishment, Lamb's Conduit Street. Lymph from these same calves was being used as the current supply at that station.

The vaccine material, which was collected 120 hours after vaccination of the calf, was procured in the following manner: the vaccinated surface of the calf was well cleansed with water, and the crusts on the vesicles wiped off with a clean towel. Next the vesicles were scraped with a sterilised spoon, and the scrapings and vesicular contents were received into a sterilised stoppered bottle of known weight.

This bottle with its contents was then immediately taken to the laboratory and the experiment proceeded with.

Experiment 1.—The vaccine material (120 hour lymph) from the bottle was divided into three equal

parts by weight, the division being effected expeditiously under cover and with sterilised instruments, so that no contamination of the vaccine might take place. Each separate portion of lymph was then rubbed up in a sterilised mortar (as before under cover) and was mixed with the chosen vehicle for its dilution. Complete distribution of the vaccine material throughout the vehicle was obtained in each instance by prolonged rubbing.

In this way:—

One part was mixed with four times its weight of 50 per cent glycerine in distilled water, so that the resulting emulsion contained 40 per cent of glycerine.

One part was mixed with four times its weight of vaseline undiluted, and the resulting emulsion contained 80 per cent vaseline.

One part was mixed with five times its weight of lanoline undiluted, so that the resulting emulsion contained 83.3 per cent of lanoline.

Immediately that satisfactory emulsions had been in each instance obtained, a definite amount of nutrient agar-agar, contained in a test-tube which had just previously been liquefied and then allowed sufficiently to cool, was inoculated with emulsion, was well shaken up, and poured on a plate. Inoculation of the agar was effected by means of a looped platinum needle—the same needle with the same loop being used for all the experiments. The number of loopfuls taken from any one emulsion was intended to be equivalent to one

PLATE III.

FIG. 3.—Photograph of agar plate prepared with vaccine material *immediately* after admixture with VASELINE.

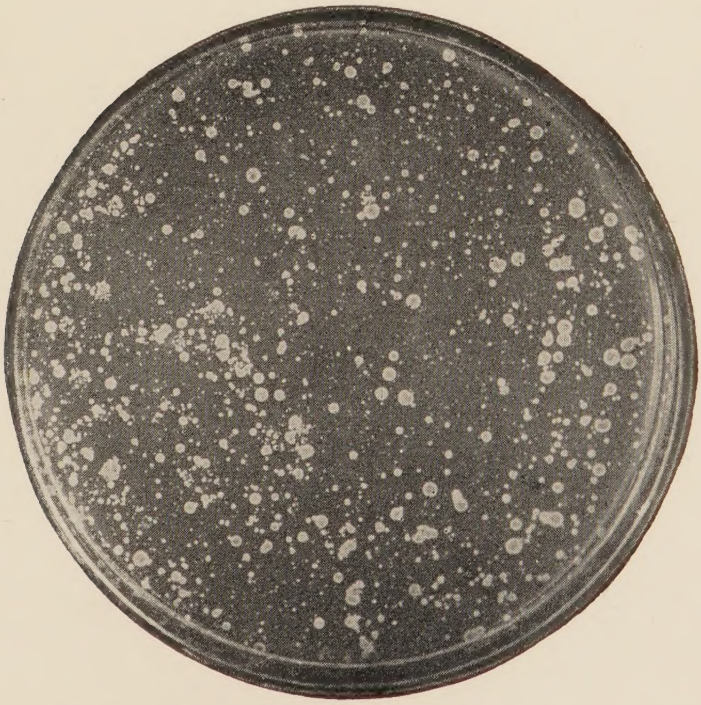


FIG. 4.—Photograph of similar agar plate prepared with vaccine material *six weeks* after admixture with VASELINE.

loopful of the undiluted vaccine material. Thus, of the diluted glycerine emulsion five loopfuls were taken, of the vaseline emulsion five, and of the lanoline emulsion six.

Three plates of nutrient agar-agar were established in the above fashion from each emulsion, the second plate being seeded through the medium of three loopfuls of material from the test-tube whence the first plate was poured, and the third plate being established in like manner from the test-tube for the second plate. The stock vaseline and lanoline emulsions were then placed in sterilised bottles duly stoppered. The stock diluted glycerine emulsion was placed in a sterilised test-tube plugged with cotton-wool. All were stored in a cool cupboard in the dark.

The several agar plates were, after solidifying, placed for twenty-four hours in an incubator at 20° C., and then for six days in an incubator at 20° C. At the expiration of that time the several plates were photographed. In this first experiment it was found, after six days' incubation at 20° C., that the first or original agar plates in all three series were so densely crowded with colonies as not to promise good pictures. Wherefore the second plates or first dilutions of the original agar tubes were alone photographed. A rough counting of the colonies on these plates, which had been established immediately after the emulsification had been effected, gave the following numbers:—

Glycerine, Plate II. . .	1000 colonies per plate
Vaseline, Plate II. . .	400 colonies per plate
Lanoline, Plate II. . .	700 colonies per plate

One week after the emulsification, further plates were established in the same manner, with the same number of loopfuls for the stock emulsions. Those plates showed an increase in the number of colonies in the samples from the vaselinated and lanolinated emulsions, but a decrease of colonies in the samples from the glycerinated emulsion. Similarly with plates made a fortnight and three weeks after making the emulsions, the colonies from the glycerinated material were fewer, whilst those from the vaselinated and lanolinated sources were much more numerous than before. At the end of four weeks none of the plates established from the glycerine emulsion showed any colonies at all, while at this date plates from the vaseline and lanoline emulsions still showed increase of colonies. At the end of six weeks plates from the glycerine stock emulsion were again found free from extraneous organisms, whereas the plates from lanoline and vaseline stock emulsions were crowded with colonies. A rough counting of these plates gave at this date:—

Glycerine, Plate I.[1] . .	0 colonies per plate
Vaseline, Plate II. . .	8,000 colonies per plate
Lanoline, Plate II. . .	10,000 colonies per plate

Experiment 2.—Scrapings of vesicles were obtained from a calf ninety-six hours after vaccination, the same precautions being taken as before. This material was weighed and divided into three equal parts. One part was emulsified with four times its weight of 50 per cent glycerine and distilled water, one part with four times

[1] Plate I.—This was so; see next page.

PLATE IV.

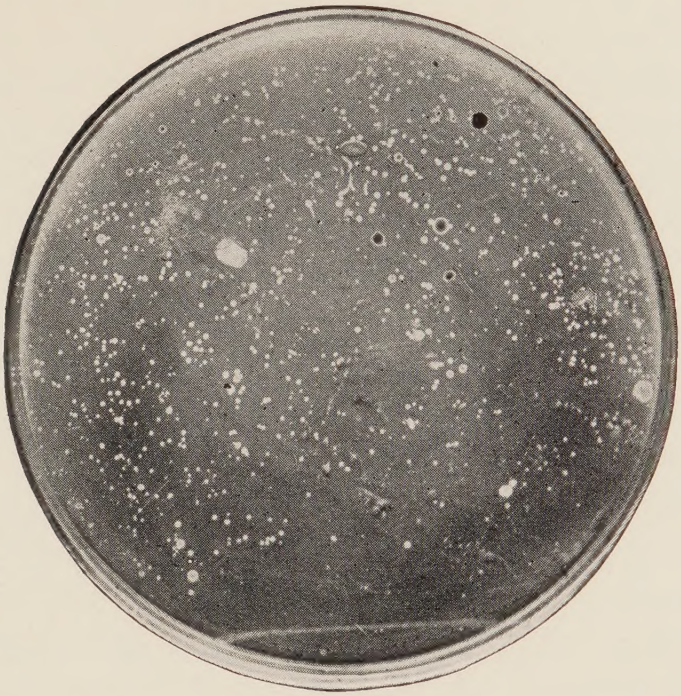


FIG. 5.—Photograph of agar plate prepared with vaccine material *immediately* after admixture with LANOLINE.

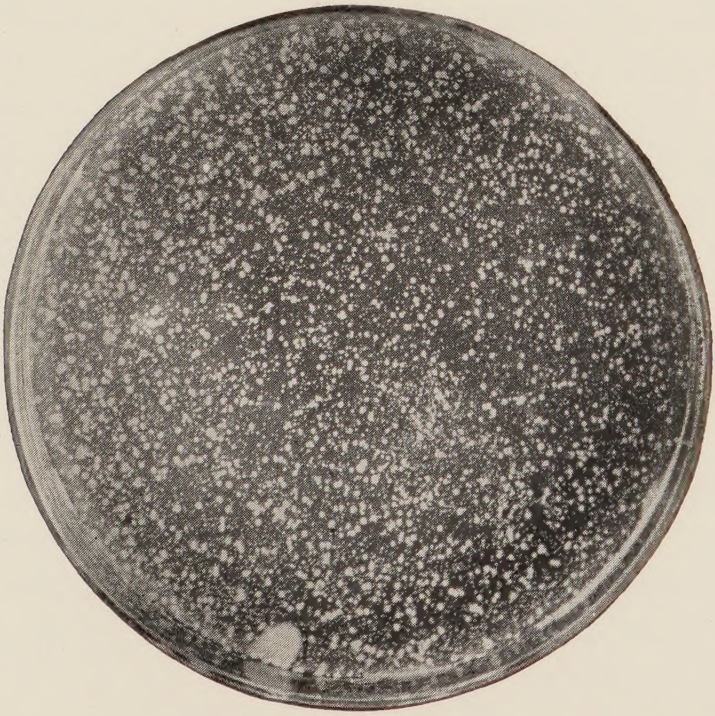


FIG. 6.—Photograph of similar agar plate prepared with vaccine material *six weeks* after admixture with LANOLINE.

its weight of vaseline undiluted, and one part with five times its weight of lanoline undiluted as in the first experiment. Agar-agar plates were established from these emulsions antecedent to their storage, and the number of colonies which appeared on the several plates at this stage were found in close agreement. The three emulsions were then placed in sterile stoppered bottles, some of the glycerinated material being also stored in capillary tubes and pipettes. It was designed to repeat the former experiment with these emulsions, and sample plates were again established from them after they had been stored a week. Again the vaseline and lanoline plates showed at this stage increase in the number of colonies, whereas the glycerine plate showed a diminution. The vaseline plate, moreover, after four days, became covered with colonies of a green mould (Penicillium glaucum), and was useless for photographic purposes.

At the end of the second week of this experiment, it was found impossible to continue it as regarded the vaseline and lanoline emulsions. These had become, in their separate sterilised bottles, both covered with a luxuriant crop of the above green mould. No such growth appeared in the bottle containing the glycerine emulsion; on the contrary, plates established from it after two and three weeks' storage showed a further diminution in the number of colonies, and at the end of the fourth week the comparison plate was altogether free from extraneous organisms.

In the foregoing experiments it will have been noted that the plates used for comparison were, in nearly

every instance, plates established from agar test-tubes directly inoculated from the emulsion. The exception to this rule was Experiment 1, in which the plates used for comparison were established from agar tubes inoculated, not directly from the emulsions, but from the primary agar tubes in which loopfuls of emulsion had been, in the first instance, distributed. In that experiment the sterile plate obtained at the end of the fourth week of storage of vaccine material *in glycerine* was the only plate of the series established directly from the original emulsion which has been photographed.

It remains to be stated in this connection that the results were "controlled" by duplicate plates established at the same time; and furthermore, that material which in plate culture showed no colonies was always tested again two or three times in similar fashion.

Experiment 3.—Scrapings of vesicles were collected from a calf 120 hours after vaccination, with the same precautions as before. These were weighed, rubbed up, and diluted with fifteen times their weight of 50 per cent sterile glycerine and water, so that the percentage of glycerine present became 46.9. One loopful of this emulsion immediately after glycerination was inoculated into nutrient agar-agar, and plates were established therewith. Plates established from the stock glycerine emulsion a week, a fortnight, three weeks, and four weeks respectively from commencement of storage, showed, as regards the first three, week by week a rapid diminution in the number of colonies which appeared after incubation in the manner described,

while the fourth was found to be quite free from extraneous organisms. Some of this glycerine emulsion, four weeks after manufacture and storage in a pipette, was used by the Government operators at the Animal Vaccine Establishment, Lamb's Conduit Street, for the vaccination of sixty-five infants. All these children's arms "took," Dr. Cory obtaining in his series of thirty-six cases 100 per cent of insertion success.

The advantages of glycerinated calf lymph may be set out as follows:—

1. By employing the method of glycerination of lymph-pulp, great increase in quantity can be obtained without any consequent deterioration in quality, the percentage of insertion success following on its use being equal to that obtained with perfectly active fresh lymph.

2. Glycerinated lymph does not dry up rapidly, as does unglycerinated lymph, thus simplifying the process of vaccination.

3. Glycerinated lymph does not coagulate, so that it never becomes necessary to discard a tube on this account.

4. Glycerinated lymph can be produced absolutely free from the various streptococci and staphylococci which are usually to be found in untreated calf lymph, and which are, under certain circumstances, liable to occasion suppuration.

5. In like manner the streptococcus of erysipelas, in the event of its having been originally present in the lymph material, is rapidly killed out by the germicidal action of the glycerine.

6. The tubercle bacillus is effectually destroyed even

when large quantities of virulent cultures have been purposely added to the lymph.

7. The possibility of inoculation of syphilis is eliminated, as the calf is not subject to this disease.

8. The necessity for collecting children together, with the attendant risk of spread of infectious diseases, or of transporting a calf from place to place, is obviated, while the danger of "late" erysipelas in the child is diminished by reason of there being no necessity to open the mature vesicles for the purpose of obtaining lymph.

9. The bacteriological purity and clinical activity of large quantities of the lymph can be readily tested prior to distribution.

10. By reason of the possibility of keeping large stocks of glycerinated lymph on hand for considerable periods of time without appreciable deterioration, any sudden demand, such as is likely to arise on the outbreak of epidemic smallpox, can be promptly met.

11. The expense of producing glycerinated lymph is proportionally small, since the amount obtainable from each calf is enormously increased.

The Preparation of Glycerinated Calf Lymph

The method best adapted for the production of glycerinated calf lymph, which shall be free from all extraneous organisms, of perfect efficacy, and yet affording material for the vaccination of many more children than the original unglycerinated calf lymph, is briefly as follows:—

The Preparation of the Calf.—A female calf of suit-

able age, about from three to six months, should be kept under observation for a week, after which, if found to be quite healthy, it may be removed to the vaccination station. It is there placed on a tilting table, and the lower part of the abdomen, reaching as far forward as the umbilicus, is shaved and thoroughly washed with a solution of carbolic acid and then rinsed with sterile water and dried with soft sterilised towels.

Inoculation of the Calf.—With a sterilised sharp scalpel incisions about four inches long and half an inch apart, parallel to the long axis of the body, are made on this clean-shaved area. The depth of the incision should be such as to pass through the epidermis and to open the rete Malpighi, if possible without drawing blood. As these incisions are made, glycerinated calf lymph, which by examination has been proved to be free from extraneous organisms, is run into them by means of a sterilised blunt instrument, and the point of the scalpel is from time to time dipped into the vaccine emulsion.

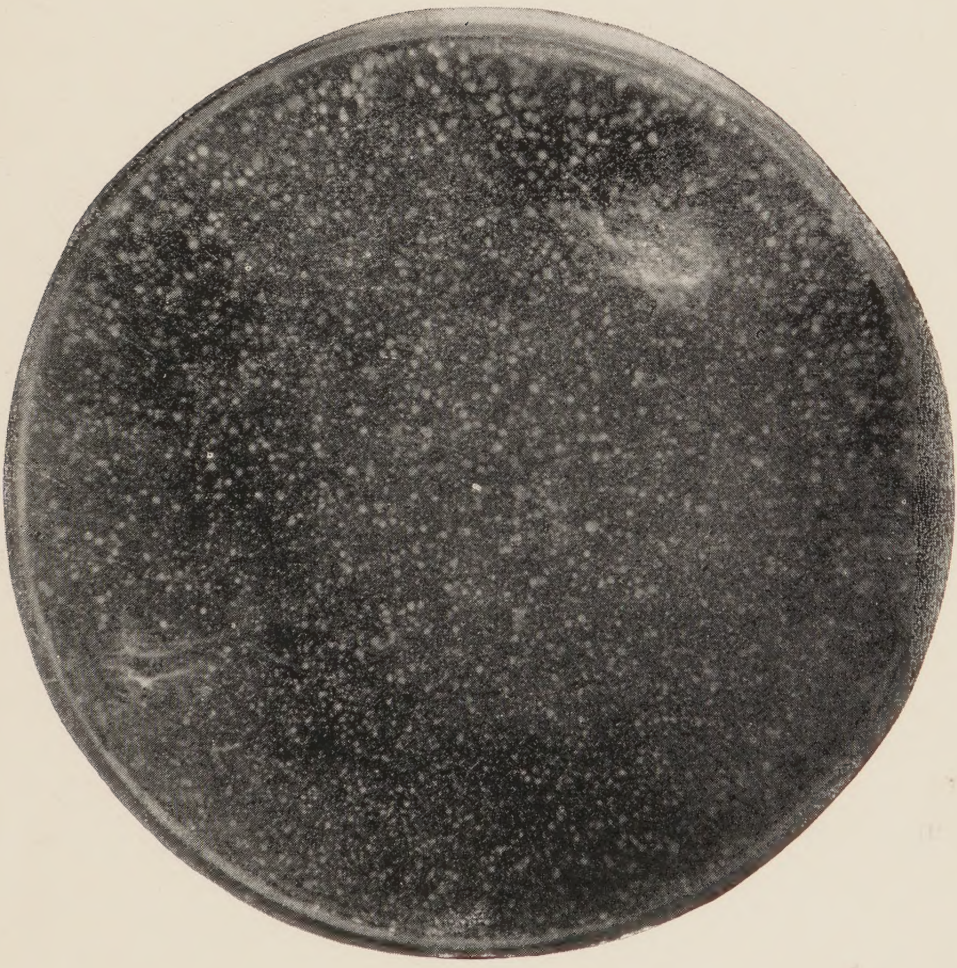
Collection from the Calf.—After five days (120 hours) the vaccinated surface of the calf is first thoroughly washed with warm water and soap, rubbed over it by the clean hand of the operator, and finally the whole area is carefully cleansed with sterile water. The remaining moisture is then removed by sterilised sheets of blotting paper. The vaccinated incisions will now appear as lines of continuous vesicles raised above the surface, each line separated from its neighbour by about a quarter of an inch of clear skin. Any crusts which appear in the vesicular lines are picked off with a blunt

sterilised instrument. The vesicles and their contents are then removed by means of a sterilised Volkmann's spoon, and transferred to a sterilised bottle of known weight. By going over the lines only once with the spoon, it is quite easy to remove the whole of the pulp without any admixture of blood. The abraded surface is carefully washed, and may be dusted over with fine oatmeal or starch and boracic powder. Subsequently, the calf is transferred to the slaughter house and the carcase is examined by the veterinary surgeon, who forwards a certificate of its condition. Should this not be satisfactory, the vaccine pulp obtained from the animal is destroyed.

Preparation and Glycerination of the Lymph-pulp.—The bottle containing the vaccine pulp is taken to the laboratory, and the exact weight of the material ascertained. A calf vaccinated in this way will yield from 18 to 24 grammes, or even more, of lymph-pulp. This material is then thoroughly rubbed up in a sterilised mortar or in a mechanical triturating machine.[1] When it has been brought to a fine state of division, it is mixed with six times its weight of a sterilised solution of 50 per cent chemically pure glycerine in distilled water. The resulting emulsion is then transferred to small test-tubes, which are then aseptically sealed and should be stored in a cool place protected from light. When required for distribution it is drawn up into sterilised capillary tubes, which are subsequently sealed in the flame of a spirit lamp.

[1] For description of instruments devised by Dr. Chalybäus of Dresden, and Dr. Doering of Berlin, respectively, see Appendix I. pp. 222 and 213.

PLATE V.

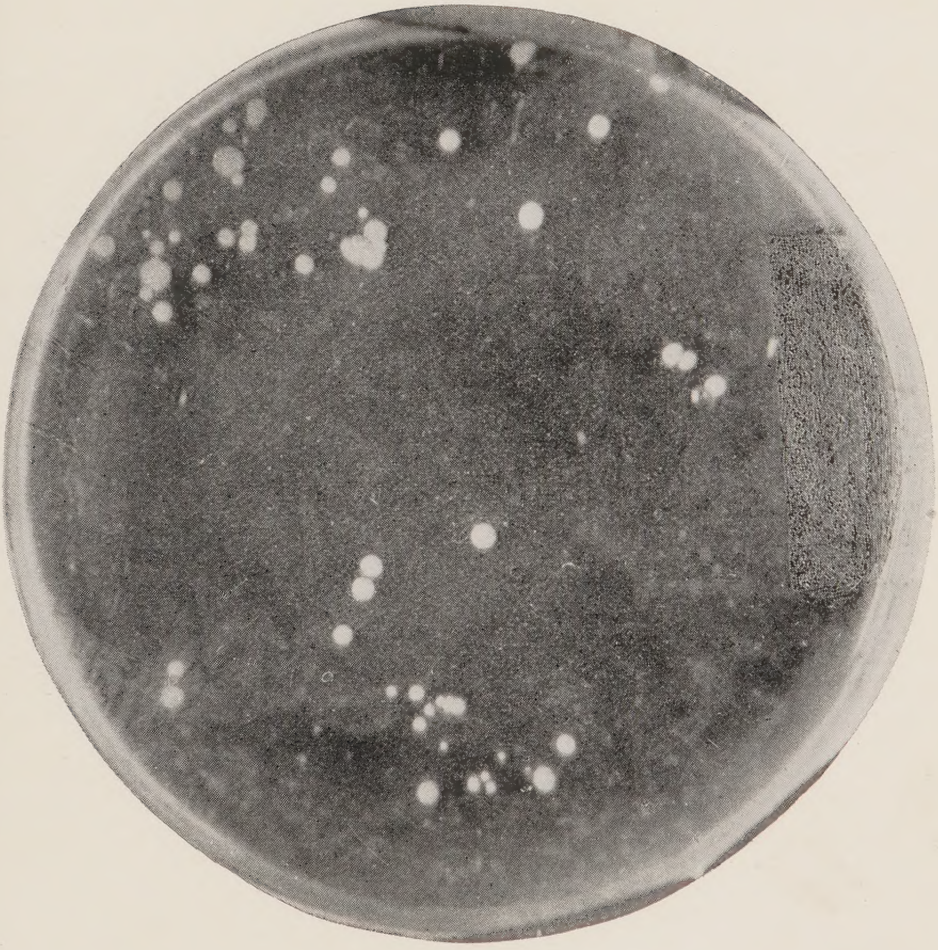


SERIES 24.—Photograph of agar plate incubated for seven days subsequent to inoculation, with one loopful of rubbed-up vaccine pulp, *immediately* after glycerination. The number of colonies is very large.

PLATE VI.

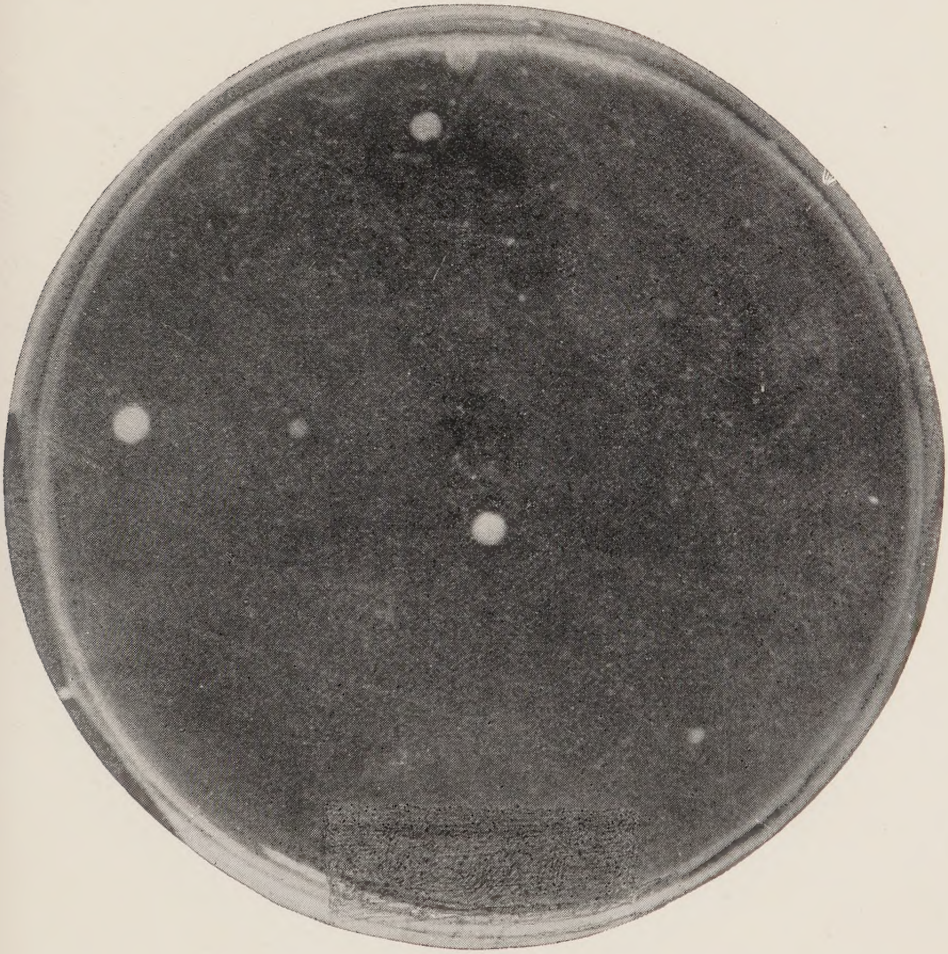
SERIES 24.—Photograph of agar plate prepared with similar quantity of vaccine material *one week* after glycerination. The number of colonies is considerably diminished.

PLATE VII.



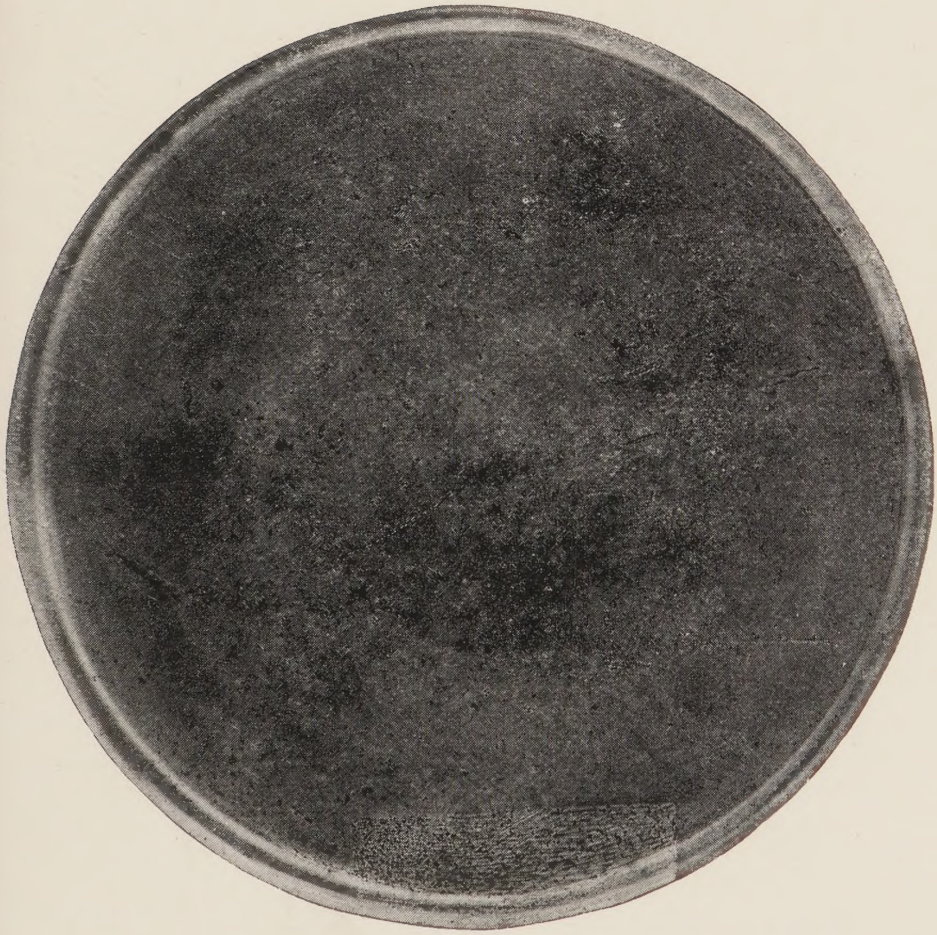
SERIES 24.—Photograph of similar agar plate, prepared with vaccine material *two weeks* after glycerination. A further decrease in the number of colonies is now conspicuous.

PLATE VIII.



SERIES 24.—Photograph of similar agar plate, prepared with vaccine material *three weeks* after glycerination. The decrease in the number of colonies is still more marked than in the former plates.

PLATE IX.



SERIES 24.—Photograph of similar agar plate, prepared with vaccine material *four weeks* after glycerination. No growth whatever has occurred.

Bacteriological Examination of the Lymph Emulsion.— As soon as the vesicular pulp is thoroughly emulsified with the glycerine solution, agar-agar plates are established from it, and, after suitable incubation for seven days, the colonies that have developed on the plates are counted and examined. Week by week this process is repeated, and invariably the number of colonies diminishes with the age of the emulsion, until at the end of the fourth week after the collection and glycerination of the lymph material, the agar-agar plates inoculated at that time show no development of colonies. The lymph is then subjected to further culture experiments, and if these results of freedom from extraneous organisms are confirmed, the emulsion is ready for distribution. The elimination of the extraneous organisms in our experiments has occurred with marked regularity at the end of the fourth week. The only exception to this rule arises when the lymph originally contained a considerable number of spores or bacilli of the hay bacillus or bacillus mesentericus. These organisms are very resistant to the action of glycerine, but if the precautions detailed are carried out in the treatment of the calf, their presence may generally be excluded.

Duration of Activity of Glycerinated Calf Lymph.— This varies in all probability with atmospheric conditions, with the fineness of division of the vesicle pulp, and above all with the condition of the calf itself. Some calves yield an excellent lymph, others a poor lymph, and the problem is to determine the value of the lymph yielded by any given calf. A lymph which was collected

and glycerinated on 13th July 1897, has since been used at intervals of from twenty-four weeks to thirty-two weeks after glycerination, for the vaccination of children. During this period, sixty-one children have been vaccinated with this lymph in five places each, with a mean insertion success of 98 per cent.[1] Thus, by the methods described, glycerinated calf lymph can be prepared which becomes freed from extraneous organisms, is available for a large number of vaccinations, at least 5000 from an average calf, and retains full activity for eight months, and will, under favourable circumstances, continue to do so in all probability for still longer periods, if necessary.

From the description which I have given, it will, I think, be obvious that the preparation and testing of glycerinated calf lymph, properly so called, is a matter requiring considerable skill and care. Sir Richard Thorne insists strongly on this fact in his introduction to our joint Report, recently presented to Government, on the administration of certain of the chief continental vaccine establishments. This Report I reproduce as an appendix, with such emendations and additions as more recent information and further experience have shown to be needed.

The following conclusions were submitted by Sir Richard Thorne in his introduction to the Report in question:—

"1. It is desirable that vaccination, both primary and

[1] This same sample of lymph (when forty-two weeks old) gave, in Dr. Cory's hands, an insertion success, based on fifty insertions, of 100 per cent.

secondary, carried out under the auspices of the Government, should be performed exclusively with vaccine lymph derived from the calf.

"2. There will probably be advantage in retaining, for a time at least, the system of calf-to-arm vaccination at the Board's Animal Vaccine Station for such parents and others as may request it, and for the purposes of comparing its results with those following the use of calf lymph preserved in one or another way.

"3. The distribution of calf vaccine from the National Vaccine Establishment should be limited to glycerinated or similar preparations of lymph or pulp material, in air-tight tubes or other glass receptacles.

"4. To give effect to the above, it will be requisite that the Board's Animal Vaccine Station should be reorganised, both as regards construction and administration. Notably will it be requisite that it should include a properly equipped laboratory under the direct supervision of a bacteriological expert."

[These recommendations are now in process of adoption by the Government, and already suitable laboratories have been secured, which have been placed in the charge of Dr. Blaxall. I trust, therefore, that the Government will shortly be in a position to supply bacteriologically pure and fully active calf vaccine lymph in any quantity that may be found desirable.]

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APPENDIX No. I

On the Preparation and Storage of Glycerinated Calf Lymph: by Drs. Thorne Thorne and Monckton Copeman

Introduction addressed by the Medical Officer to the Right Honourable Henry Chaplin, M.P., President of the Local Government Board

Sir—Shortly after the issue of the Report of the Royal Commission on Vaccination last autumn, I received your instructions that I should, together with Dr. Monckton Copeman, visit certain cities in different countries on the Continent of Europe, with a view of obtaining information as to the methods adopted, by the respective authorities and others concerned, in the distribution of vaccine lymph derived from the calf, more especially in reference to the preparation, storage, and distribution of glycerinated calf lymph.

The necessary visits were commenced in December of last year, but owing to the fact that in some of the countries to be visited public vaccination is practically limited to certain months of the year, commencing with the spring, it was found impossible to complete the inspections until quite recently. In eliciting information as to the methods adopted in each of the countries visited, we held especially in view two points to which the Royal Commission gave prominence

in their Report. The first of these—one which the Commissioners put "in the forefront," is the recommendation in section 437 of their Report "that parents should not be required to submit their children to vaccination by means of any but calf lymph." The second, which is referred to in section 448, is concerned with experiments which Dr. Copeman made and announced to the International Congress of Hygiene which met in London in August 1891, as to the effect of the storage of vaccine lymph in glycerine. "The conclusions at which he arrives," say the Commissioners, "are that the addition of glycerine, whilst it leaves the efficacy of the lymph undiminished, or even increases it, tends to destroy other organisms"; and they add that "The question is one a further investigation of which is obviously desirable."

The places visited by us were Paris, Brussels, Berlin, Dresden, Cologne, and Geneva; and in submitting to you an account of the operations which came under our notice in each of these cities, I beg leave to make the following observations.

It will be noted that in each of the countries concerned, vaccination with calf lymph has become the habitual, if not the universal practice. In some, indeed, we were informed that, although vaccination with humanised lymph is not definitely prohibited under any statute or regulation, yet resort to such lymph by any medical practitioner having official responsibility to the Government is altogether discouraged.

In only one of the places visited—namely, Paris—did we find that vaccination was carried out under official sanction with crude calf lymph, and even there the process was limited to vaccination direct from calf to arm, all lymph stored for distribution being glycerinated calf lymph.

The circumstances of Paris in the matter of vaccination direct from the calf deserve consideration, because they have a certain similarity to those which may be encountered in this country, in case the use of calf lymph should become habitual. Thus, we were informed that if vaccination had, during past years, been limited to the use of glycerinated calf lymph, stored in tubes or otherwise, it would have been found difficult, amongst certain classes of a population which heretofore had only known of stored humanised lymph, to convince persons whose vaccination it was desirable to secure that the lymph proposed to be used on them was really calf lymph, not humanised lymph. In order to ensure confidence in this respect, it had been the practice to convey calves to the vaccination stations, or to districts infected with smallpox, and to perform the vaccination direct from calf to arm. Perusal of the account which we give as to this practice in the hands of MM. Chambon and Ménard will show that it has been brought to a position of very considerable efficiency.

In all the other countries visited we found that, acting on the indications announced by Dr. Copeman in 1891, the Governments and other authorities concerned had made sustained investigation as to the preparation, storage, and use of glycerinated calf lymph, and had gradually come to adopt that preparation of lymph for official, and in some cases for all, purposes throughout their jurisdictions. Thus, in Germany we were informed that the system of vaccinating direct from the calf had come to be abandoned as completely as that from arm to arm, the use of glycerinated calf lymph having become general throughout the Empire.

The reasons for this change have been two. The governing reason has been the confirmation by competent bacteriologists of the results obtained by Dr. Copeman, to

the effect that, by the admixture to calf lymph of a 50 per cent solution of pure glycerine in sterile water, and by subsequent storage of the lymph material in tubes, under due precautions, for a term of several weeks, the preparation remained quite active as vaccine, whilst a very remarkable germicidal effect was produced on extraneous micro-organisms in the lymph, even including certain pathogenic organisms which had been purposely added to the lymph material. The second reason was that, by reason of the admixture referred to, the amount of vaccine procurable from a given calf could be greatly, even enormously, increased, and that, within certain wide limits, this could be done without interfering with the insertion success following on the use of the lymph. At the Board's Animal Vaccine Establishment it has hitherto not been deemed necessary, nor even expedient, to make one calf serve for more than some 200 to 300 vaccinations. It is no unusual thing abroad to provide from a single calf an amount of glycerinated lymph that shall serve for from 4000 to 6000 vaccinations, and in Berlin we were assured that the glycerinated lymph which was prepared in our presence from one calf would suffice for no less than 15,000 vaccinations. We brought some of this Berlin lymph to England, and it was used for the purposes of vaccination at intervals of nine, eleven, and thirty-seven days after its collection, with the result that in 76 vaccinations performed, in each case by five insertions, its use resulted in a mean insertion success of 92.0 per cent. Storage of this particular sample for a much longer period did not give satisfactory results. With other preparations of glycerinated lymph, diluted to about one-third of the amount of the Berlin sample, and which were used in England at intervals varying from seven to thirty-one days after collection and preparation, the

insertion success reached 97, 98, and 99 per cent; and in the case of 111 vaccinations, all effected with two other supplies of glycerinated lymph, used at intervals of from seven to thirty days after preparation, the success reached 100 per cent, every insertion of lymph being followed with success. In all these cases the vaccination was performed by means of five insertions.

In every instance we found that the work of collecting, preparing, and storing the glycerinated lymph was carried out with the greatest care; a condition of scientific cleanliness was especially aimed at, and a laboratory, fitted with bacteriological and other scientific apparatus, always formed an essential part of the vaccine institution. The extent to which the desired end of freedom from extraneous impurity was attained depended largely on whether a first attempt to adapt an existing calf station or similar establishment to its new purposes had been maintained, or whether it had been abandoned in favour of an institution constructed especially for the purposes of that which is in the main scientific laboratory work. Several of the stations which we visited are already under condemnation, because of the difficulty of ensuring that freedom from extraneous micro-organisms which should be aimed at during the preparation of the lymph supply; the Cologne station is one of the newest, and may well serve, in its main features, as a type of that which should be aimed at.

The condition of scientific cleanliness to which I have referred extended to such matters as the following: (*a*) the construction and administration of the stabling for the calves; (*b*) the means for washing or bathing calves before their vaccination; (*c*) the construction, cleansing, etc., of the operating rooms; (*d*) the cleansing of the vaccinated surface of the calf with germicidal preparations and sterilised

cloths before collection of the lymph; (*e*) the use of clean sterilised outer garments by all officials concerned in the processes carried out; (*f*) the sterilisation of all instruments, etc., employed; and (*g*) the carrying out of the process of admixture of the lymph material or pulp with glycerine, as also its preparation and storage under conditions of laboratory freedom from extraneous organisms.

Further, we found that it was an invariable practice at the stations visited on the Continent not to issue any lymph until a report had been received from a veterinary surgeon, after slaughter of the animal, as to the freedom of the calf furnishing it from disease; in brief, the lymph issued is that of healthy calves only. This practice is mainly with a view of avoiding all risk of conveying tuberculosis along with calf lymph; though such risk would, under any circumstances, be a very remote one, seeing that tuberculosis is extremely rare in young bovine animals, and seeing also that the tubercle bacillus, when experimentally added to a mixture of lymph and an aqueous solution of glycerine, rapidly loses its vitality.

The information which we obtained in the course of our visits does not profess to be complete. Much remains to be ascertained by careful scientific research, in order to learn what are the precise conditions under which glycerinated calf lymph can be prepared and stored, so as to secure to the utmost freedom from extraneous, and especially from pathogenic micro-organisms, whilst at the same time retaining to the utmost the undiminished protective value of the lymph material against smallpox. We learned that in every country visited, further research is being made in this direction, and in Germany a special commission of medical and bacteriological experts has been appointed by the Government to study and report upon the subject.

But the information which is now available in this country, and that which, during the course of our visits abroad, was placed at our disposal with a readiness and a courtesy which calls for an expression of the fullest acknowledgment, suffices to enable me to submit the following conclusions for your consideration :—

1. It is desirable that vaccination, both primary and secondary, carried out under the auspices of the Government, should be performed exclusively with vaccine lymph derived from the calf.

2. There will probably be advantage in retaining, for a time at least, the system of calf-to-arm vaccination at the Board's Animal Vaccine Station for such parents and others as may specially desire it, and for the purposes of comparing its results with those following the use of calf lymph preserved in one or another way.

3. The distribution of calf vaccine from the National Vaccine Establishment should be limited to glycerinated or similar preparations of lymph and pulp material, in air-tight tubes, or other glass receptacles.

4. To give effect to the above it will be requisite that the Board's Animal Vaccine Station should be reorganised, both as regards construction and administration. Notably will it be requisite that it should include a properly equipped laboratory, under the direct supervision of a bacteriological expert.—I have the honour to be, sir, your obedient servant,

RICHD. THORNE THORNE.

July 1897.

APPENDIX No. I—*Continued*

Report on the Results of an Inspection made by Dr. R. Thorne Thorne and Dr. S. Monckton Copeman as to Vaccination Arrangements adopted in certain European Countries with special reference to the Preparation and Storage of Glycerinated Calf Vaccine Lymph. (Prepared by Dr. S. Monckton Copeman.)

Paris

At Paris we spent several days in inspecting the manner in which the work of obtaining, preparing, storing, and distributing calf vaccine lymph is carried out at the Institut de Vaccine Animale and at the Académie de Médecine respectively.

The Institut de Vaccine Animale

This establishment is carried on by M. Chambon and Dr. St. Yves Ménard. It is, practically, a private establishment, although the municipality of the city of Paris contract with the directors to carry out all such public vaccinations, within their jurisdiction, as may be necessary.

The Institut de Vaccine Animale, which is situate in the Rue Ballu, consists of what was originally a dwelling-house, with a courtyard opening to the street alongside, and a stable behind, the portion of the courtyard immediately adjoining the stable being covered over and provided with sliding doors, so as to form an operating room when neces-

sary. Rooms in the dwelling-house on the ground floor are set aside as waiting and operating rooms in which persons are vaccinated direct from the calf, while on the first floor are other rooms in which the calf lymph is manipulated, placed in sealed tubes, stored, and distributed.

Stable.—This is a building about 18 feet square, which contains stalls for ten calves. Each stall is somewhat narrow, but we were informed that this was advantageous, as the animal was thus kept more quiet than would otherwise be the case. Attached to the halter of each animal is a large iron ring, which runs on a vertical iron rod let into the wall of the building, above and below. This arrangement was devised in order to prevent the calves from being able to lick the inoculated area of their body, while, at the same time, it does not prevent them from lying down. The floor of the stable, which is formed of roughened bricks, slopes slightly to a shallow drain on either side of a footway between the two rows of stalls. The building is heated artificially by means of hot-water pipes, and its ventilation is aided by means of an extraction shaft containing a lighted gas jet. At the time of our visit the temperature was about 15° C. The walls are covered with glazed tiles, and the floor is laid with bricks which are impervious to moisture. Both walls and floor are occasionally washed down by the aid of a spray of a solution of perchloride of mercury.

Calves.—These animals, which appear to be in every respect well suited for the purpose of lymph propagation, are of a special breed, and are obtained from the Department of Corrèze in the southern half of France. They are all of a uniform reddish-brown colour, with fairly long, soft hair. Their skin, when exposed by shaving, is seen to be particularly smooth and supple, and it varies in colour from

pink to a pale shade of brown. For the purpose of keeping free from urine the straw-bed which comes into contact with the surface of the body operated on, only cow-calves are employed, which vary in age from four to six months, the average being about eighteen weeks. Prior to being brought to the Institut stable, they are kept for about twelve days in a quarantine shed in the outskirts of Montmartre. They are all weaned at the age of two months, and they receive no milk or eggs while at the quarantine shed or at the Institut stable, the food of each calf consisting of—

One litre of crushed oats,
Two litres of bran, and
Three kilos of hay

twice daily, at about 6 A.M. and 4 P.M. At both places water is supplied from the town mains.

The calves cost, on an average, about 125 francs, with an additional 22 francs for travelling, etc., making 147 francs in all. After use, they are sold to a butcher, at a loss of from 30 to 40 francs.

On the day after arrival at the stable the calves are vaccinated. Injection of tuberculin is not employed, as the directors consider this unnecessary, in view of the fact that immediately after collection of the lymph each calf is sent to the abattoir and slaughtered under the personal supervision of a medical man, whose report is awaited before any of the lymph is distributed for use. In the event of any signs of tuberculosis being found, the whole of the lymph derived from this particular animal would be destroyed.

Vaccination of Calves.—For this purpose one or more large tubes of glycerinated lymph, which have been kept for at least a month subsequent to its collection and storage, are employed, the directors considering that better results

are thus obtained than if the operation were carried out directly from calf to calf.

For the purposes of the operation the calf is strapped to a tilting table somewhat similar in design to those employed in this country, but the right hind leg of the animal is not elevated, and in consequence the mammary region is not exposed, indeed it is not utilised for inoculation.

To prepare a surface for the insertion of the lymph the right side of the animal is thoroughly scrubbed with soap and hot water, and then shaved over an area extending between the internal edges of the fore and hind limbs, and from some 4 or 5 inches below the spinal ridge to the umbilicus. The shaved area is next washed with soap and hot water, then with a hot solution of boracic acid, and, finally, with plain hot water. It is afterwards dried with clean soft cloths.

A number of superficial incisions, each about 1 inch long, are then made in a direction at right angles to the long axis of the body, and about a couple of inches one from another. The incisions of the several rows are made *en échelon*. The lancet employed for the purpose has a spear-headed blade, this shape being specially recommended by M. Chambon. Over each incision a drop of glycerinated lymph is allowed to fall from a glass tube, and the drop is rubbed in with the flat portion of the blade of the lancet. The process is carried out by one of the laboratory servants, and is a somewhat lengthy one.

When the lymph has dried, the calf is removed from the table and taken back to its stall.

Collection of Lymph.—The vaccine material is always collected on the sixth day. The calf is once more placed on the table; or, if material is required for immediate use only, it is usually allowed to stand. The vaccinated area

is washed with warm water and dried with clean soft cloths. Each vesicle is now clamped separately, and the crust first removed with a lancet, which is then wiped on a cloth pinned to the front of the clean cotton blouse which the operator has previously donned.

The vesicle is then thoroughly scraped with the edge of a somewhat blunt lancet, and the resulting mixture of lymph, epithelial tissue, and blood is transferred to a small nickel crucible set in a wide wooden stand on a table close to the operator. The crucible is provided with a cover which is kept over it except at the moment when a further addition is made to its contents. The collection of all the vesicular material obtainable from one calf appears to take about three-quarters of an hour.

To the pultaceous mass contained in the crucible there is added about an equal quantity of glycerine, which was described to us as "doubly rectified," but which appeared to be of very thin consistence as compared with the best English glycerine. No accurate measurement of the quantity employed is made.

The mixture of pulp and glycerine is triturated in a mixing machine devised by Dr. Chalybäus of Dresden, the particular one that we saw being driven by a small electric motor. (A description of this machine by Dr. Chalybäus himself will be found on pp. 222-224.)

The mixture, having thus been rendered thin and homogeneous, is received in a clean sterilised nickel crucible placed beneath the machine, but with a view of still further improving its appearance and of removing any extraneous matters, such as hairs, it is afterwards pressed through a small brass-wire sieve, consisting of extremely fine gauze, into an agate mortar. This is done by means of a bone spoon, and there is left on the surface of the gauze nothing

but a very small quantity of epithelial tissue, together with a few hairs. The mixture is further triturated in the mortar with an agate pestle, and is then ready for filling into the tubes in which it is distributed.

Storage of Lymph.—The lymph material is next drawn up into a sterilised glass syringe provided with a metal nozzle of such a size that the tubes to be filled can easily be fitted over it. Slight pressure on the piston of the syringe causes sufficient lymph to enter the tube. Each tube is filled about two-thirds full, and is then placed on a porcelain tray, pending its being sealed. This is done without delay by means of a blow-pipe, the air blast of which is provided by means of a pressure apparatus in one corner of the room. The tube is first tilted until the column of lymph occupies the central portion, and it is then held in a horizontal position, while each end is successively placed in the blow-pipe flame, and, when sufficiently melted, drawn out by means of a pair of forceps, and so sealed.

Distribution of Lymph.—The tubes when sealed are placed with a small surrounding of cotton-wool in small light metal tubes provided with a tightly-fitting cover. These cases, if sent out singly, are fitted into a block of wood grooved on one side, being kept in position by a paper label, which is gummed round the block, and which has on one side space for postal address and stamp, and, on the other, directions for use.

A register is kept of persons to whom supplies of lymph are sent, and of the calf from which each supply is derived.

Académie de Médecine

We also visited the vaccine station of the Académie de Médecine, of which Dr. Hervieux is the director. The general principles on which this institution is conducted are

so similar to those which have been set out at length in the description of MM. Chambon and Ménard's establishment, that it is unnecessary to enter into similar details again. It should be mentioned, however, that at the Académie de Médecine the calves employed were not of the same breed as those used at the Institut Vaccinal, neither did the vesicles which we had an opportunity of seeing, on a single visit, appear to be quite equal in character to those which we observed at the latter institution.

Calf-to-Arm Vaccination at Nanterre

While in Paris we were afforded an opportunity of seeing an extensive series of vaccinations and revaccinations, carried out directly from calf to arm, at the Nanterre House of Detention. The calf, which arrived at the establishment in a closed van, was brought from the vaccine institute of MM. Chambon and Ménard, and the vaccinations were carried out by M. Chambon and by one of the medical officers to the establishment.

The operations were performed in a small square room, having a door at each of two opposite angles ; opposite the door of entry the calf was tied up to a post, and, in front of and facing it, an assistant took up his position in order to collect the lymph, by scraping slightly one of the vesicles on the calf's abdomen, to which compression forceps had been applied. On his right hand were placed a tray containing lancets and compression forceps, a rack for holding charged lancets, and a glass bowl containing a pad of cotton-wool floating in perchloride of mercury solution. On either side of him, and so arranged as to be able with ease to reach the tray, sat one of the two operators, each of whom had placed in front of him a chair for the vaccinee.

The male inmates, each with his shirt sleeve on the left arm rolled up to the shoulder, were admitted by the door opposite the calf, an assistant, wearing an ambulance badge, giving the upper part of each person's arm a brisk rubbing with a cloth soaked in boracic acid solution as he entered, the arm being afterwards dried with another cloth. Two or three warders were also in attendance, who so directed the stream of inmates that each operation chair was refilled as soon as vacated.

On an inmate seating himself, the operator took a charged lancet from the rack, with which he made three punctures in an oblique direction, just beneath the skin of the upper arm. The lancet was then dropped into the glass bowl containing the disinfectant, from which, in turn, it was removed by the assistant, who wiped it on a previously sterilised cloth, and the instrument was then recharged.

One assistant was thus able to keep the two operators supplied with charged lancets as rapidly as they were required, and it will be obvious that the whole scheme of operation had been well devised, and was skilfully and expeditiously carried out, when we mention that during our visit no less than 480 vaccinations were performed in the short space of thirty-nine minutes. Most of the vaccinations were revaccinations, some of the inmates having been submitted to the same operation on the occasion of previous admissions to this or similar institutions.

Arrangements for Gratuitous Domiciliary Vaccination in the City of Paris

As already stated, MM. Chambon and Ménard are entrusted, by the municipality of Paris, with the carrying

out of arrangements for the vaccination and revaccination of the inhabitants; and on receiving information of the occurrence of smallpox in any part of the city, they make domiciliary visits for the purpose of offering vaccination to persons who may be unable or unwilling to attend the public stations.

The notifications as to the existence of smallpox are at once sent on to MM. Chambon and Ménard, who then make arrangements to attend at the house or neighbourhood in question on the following day; but in the meantime the day and time of attendance are intimated to the inhabitants by means of printed cards having blank spaces for the insertion of the necessary particulars. In Paris each "house," in most of the quarters occupied by the poor and the working classes, is made up of a series of flats, which, again, are subdivided up into dwellings of one or more rooms, the number of persons inhabiting each house being, therefore, much greater than is the case in this country. Each such "house" has a porter's lodge at the entrance, and it is outside this lodge that the notice previously mentioned is displayed, and it is in this lodge also that, at the pre-arranged time, the vaccinations and revaccinations are generally performed. For these operations calf lymph is invariably employed, the process being carried out direct from calf to arm, a previously vaccinated calf being sent to the house from the Institut Vaccinal in a specially constructed van. Occasionally, from want of space in the porter's lodge or other reasons, the calf, after being removed from the van, is allowed to remain in the street, its halter being held by an attendant, while another assistant, taking his seat on a camp stool, proceeds to collect lymph from the inoculated area of the animal's side and abdomen, with the aid of clamp forceps

and lancet. Where such procedure is considered necessary, the persons requiring vaccination, whether infants or adults, also are brought out into the street, and the extraordinary spectacle may be witnessed of vaccinations being carried out by the medical staff surrounded by an interested crowd of sightseers.

MM. Chambon and Ménard attach much importance to this organisation and practice. They say that, under the immediate influence of existing smallpox, large numbers are willingly submitted, both to primary and secondary vaccination, who would otherwise escape; and they are of opinion that certain classes who might object to be vaccinated with lymph from an unknown source, find all their objections on this score removed when they actually see the calf which serves as vaccinifer.

We were supplied both by Dr. Hervieux and by MM. Chambon and Ménard with samples of the glycerinated lymph, which had been collected and prepared on the occasion of our visits. That which was obtained from the Académie de Médecine was collected on 10th December 1896. It was used by Dr. Cory at the Board's Animal Vaccine Station for the vaccination of twenty-seven children on 29th December. All the cases were, as usual, vaccinated by means of five insertions of lymph, and the insertion success obtained was 99.3 per cent.

That obtained from MM. Chambon and Ménard was collected on 8th December 1896; it was used by Dr. Cory on 22nd and 31st December, and on 7th January 1897, for the vaccination of ninety-six children, and every one of the five insertions succeeded in every child.

[It should be stated, however, that these specimens of lymph obtained from Dr. Hervieux, and from MM. Chambon and Ménard, respectively, were all found to contain large

numbers of extraneous microbes when examined by the method of plate-cultivation, even after the lapse of four weeks from the date of preparation. This fact is, no doubt, to be accounted for by reason of the percentage of glycerine employed not being sufficiently large. MM. Chambon and Ménard informed us that they do not profess to attain bacteriological purity in the lymph distributed from their establishment.—S. M. C.]

Brussels

At Brussels the propagation, storage, and distribution of calf lymph is carried out at the École Vétérinaire, under the supervision of Professor Degive, the director of that establishment.

The building set apart for the calf lymph station contains the director's room, a distributing room, an operating and preparation room, and two stables. As, however, we were informed by Professor Degive that the accommodation at present provided is regarded as very insufficient for the purpose, and that a new vaccinal institute is about to be erected, it would serve no useful purpose to enter into a detailed description of the present building.

Stable.—The stable, which is a detached building, contain stalls for six calves, three on either side of a central footway, and the stalls are so arranged that a space is also left between them and the side walls of the building. The stalls are very narrow, and, at the end furthest from the central passage, have two iron uprights fastened to the sides of the stall. Iron rings, which are attached to the animal's halter by means of steel clips, slide up and down the uprights. This arrangement permits the calf to stand up

or lie down, but prevents all possibility of its licking the inoculated portion of its body.

The stable is warmed by an iron stove, the temperature at the time of our visit being 15° C. It is ventilated by windows opening inwards in the upper part of the two outside walls, and the removal of vitiated air is further facilitated by four outlet ventilators, each about 6 inches square, and placed just above the floor level, the up-draught being aided by means of gas jets in the outlet shafts.

Calves.—The calves are not of any special breed, and those that we saw did not seem to be so well suited for the purpose of lymph propagation as certain of those thus employed at the Institut Vaccinal in Paris. Another point of difference is that at Brussels male animals are used exclusively; Dr. Degive believing that the finest vesicles are obtained on the scrotum. For the first four days after their reception the calves are kept in a separate "quarantine" stable. On the day prior to vaccination they are swung, by means of a belly-band and an arrangement of pulleys, into a wooden, zinc-lined, tank bath, capable of containing sufficient water to cover nearly the whole body. The temperature of the water is kept at about 30° C. In this bath the calves are scrubbed all over with soft soap. After removal from the bath, the skin is thoroughly dried with cloths, and the animal is then placed in the stable adjoining the operating room.

Each calf is injected with $1\frac{1}{2}$ cubic centimetres of tuberculin on the day prior to vaccination, but Dr. Degive considers this an unnecessary precaution for the reasons that (*a*) tuberculosis is very rare in calves, and that (*b*) no lymph is distributed until the animal from which it was obtained has been slaughtered, and necropsy has made it

certain that the animal was not the subject of tuberculosis. In the event of tubercle being found, the lymph would be destroyed.

The *age* of the calves employed averages from ten to fourteen weeks, but animals four months old are sometimes used.

The *food* of the calves consists principally of milk and eggs, each calf receiving, in twenty-four hours, 12 litres of milk and four eggs, together with a little hay, which is placed in each stall for the animal to eat if it is so disposed.

Inoculation of Calves.—The calf is fixed, by means of ropes, to a tilting table of somewhat primitive construction, the right hind limb being elevated, as is usually done in England. A leather blinker is also fastened over the head. The right side and the abdomen are washed with soap and water with which lysol is mixed, and the surface is then shaved. The skin is afterwards washed with warm boracic acid solution, then with hot boiled water, and it is subsequently dried with cloths which are sterilised by steam just previous to use. All instruments are also boiled in a solution of boracic acid. The lancets employed are similar in form to those devised by M. Chambon, while the compression forceps appeared to be of somewhat old design and needlessly heavy.

The operator and his assistants all wear white blouses, which, just previous to use, have been sterilised in an autoclave.

Incisions, about 2 or 3 inches in length, are made at right angles to the long axis of the body, all over the shaved area of the skin, and also on the scrotum; the average number for each calf being about 150. The incisions, which in each case are double, are made with a dry lancet, and are placed *en échelon*, and about a couple of inches distant one from another.

The lymph employed for vaccination is kept in stock for, at least, six weeks previous to its use ; it consists of vesicular pulp which, at the time of collection, is simply mixed with twice its weight of glycerine and is then kept in a glass tube, the mouth of which is closed with a cork fixed with paraffin until required. Just before it is needed for inoculation of a calf the pulp is ground up in a small agate mortar with a further small quantity of glycerine. The resulting emulsion is well rubbed into each separate incision on the skin by means of a thin ivory instrument resembling a small paper-knife ; the edge being passed up and down each incision several times.

Collection of Lymph.—On the sixth day the calf is again fixed on the operating table, and the vaccinated area is once more washed with warm water or sterilised salt solution and dried with sterilised cloths. The lymph required for stock purposes is then first collected. For this purpose compression forceps are applied to each vesicle separately, and the crust is first carefully removed with the edge of a lancet. These crusts are collected in a watch-glass, and are employed for the vaccination of children. The vesicular pulp is next removed by scraping with the lancet, and the material is collected in another watch-glass or Petri dish and weighed. Glycerine is added to it from a stock bottle to the extent of about twice the weight of the pulp, but the amount is only roughly estimated, no actual measurement or weighing being deemed necessary. The pulp and glycerine are stirred together, and are at once placed in a glass tube of such a size as to ensure its being almost entirely filled with the material available ; it is then fastened down by means of a glass stopper or cork, without further manipulation.

When sufficient pulp for stock purposes has been

obtained, the remainder of the vesicles are scraped off with a Volkmann's spoon, and the material is mixed, as before, with glycerine, without trituration.

The glycerine employed was stated to be of English manufacture, but was much thinner than that usually sold in this country, giving the impression, indeed, that it had been considerably diluted with water. It is sold as being "chemically pure."

Storage and Distribution.—Just as is the case with what is termed the "stock" supply, this material is ground down in a mortar, with more glycerine, before being distributed for use.

According to the amount required, the emulsion is either stored in tiny stoppered bottles, which are supposed to contain enough material for 25, 50, or 100 vaccinations; or, when a less quantity is desired, the material is placed in a slight excavation on the surface of a small glass plate about 1 inch square, and a plate of a similar size, but not hollowed out, is slid over it. The edges are sealed with paraffin, and the whole is wrapped round, first with tinfoil, and then with paper.

The small bottles are fitted into blocks of wood, bored with holes for the purpose, in order that the parcel may go safely through the post.

To each package is attached, by string, a doubled card, which can be addressed outside, and which, inside, has spaces for particulars as to number of vaccinations carried out, the number of insertions in each case, and the amount of success which results.

The lymph which was supplied to us by Professor Degive was used for certain bacteriological investigations; hence we have no record as to its success when used for the purposes of vaccination. But, from a number of returns made

by different vaccinators to Professor Degive, we found that these showed a high percentage of insertion success.

While at Brussels, we also visited the Municipal Vaccination Station, of which Dr. Janssens, the Medical Officer of Health, is director.

We were informed that the station, which consists of a waiting and an operating room, is open daily, but that, practically, no children are brought for vaccination during the winter months. This was unfortunately the case at the time of our visit, so that we had no opportunity of seeing the work in actual operation.

The lymph employed is received in small glass-stoppered bottles from Professor Degive, of the École Vétérinaire.

Berlin

The Animal Vaccine Establishment at Berlin, of which Dr. Schultz is the director, is situated in the Central Meat Market, on the outskirts of the city.

The station consists of three parts connected with each other: (1) A large stable containing stalls for the calves; (2) a work-room fitted with two tilting tables, somewhat similar to those in use in England, on which the calves are vaccinated, or the "lymph" collected; and (3) the director's room, in which the lymph is triturated, glycerinated, and stored. This room contains cupboards and benches, and is fitted with all the necessary bacteriological apparatus, glassware, and instruments; the latter being made entirely of metal, so as to admit of their being readily sterilised.

Calves.—Cow-calves are almost invariably used, as less likely, when they lie down, to foul with their urine the vaccinated area of the abdomen than are males. The calves employed are usually from about six weeks to three

months old. They are received forty-eight hours before they are required for vaccination, and are at once injected with half a gramme of tuberculin. If their temperature should rise above 41° C. during the next twenty-four hours they are not employed for the production of lymph. The calves are fed on a mixture of milk, eggs, and corn-flour, of which the milk is always sterilised prior to use.

Vaccination of Calves.—When placed on the table, the abdomen is shaved from the vulva to the umbilicus, and a portion of the inside of each thigh is also shaved. The surface of skin thus exposed is carefully scrubbed with soap and water, washed with a solution of corrosive sublimate 1-1000th, and then again washed with boiled water. The operator also washes his hands carefully, using carbolic soap, and before commencing to operate puts on a white cotton blouse over his coat. The calf's skin having been dried with a clean towel, long parallel incisions are then made over the whole length of the abdomen, and also over the shaved portion of the thighs. These incisions, which are made with a blunt knife, so as to draw as little blood as possible, are hardly a quarter of an inch apart, and are about 18 to 24 inches in length on the surface of the abdomen. If any blood appears along the line of the incisions it is removed by means of sterilised blotting-paper. A few grammes of stored glycerinated lymph, prepared some weeks or months previously, are next poured on the abdomen and spread over the incised lines with the back of a scalpel.

Collection of Lymph.—On the fourth or fifth day (96 or 120 hours after vaccination) the calf is again placed on the table. After a thorough cleansing of the skin in the same manner as before, absolute alcohol is poured over the vaccinated area. When the alcohol has evaporated the surface is treated with ether, which is supposed to exert

a bactericidal, in addition to its anæsthetic action. The use of alcohol and ether has, however, been discontinued since the date of our visit, soap and water merely being now employed. Then the skin is put on the stretch and scraped, in the direction of the incisions, with a rather blunt "Kartoffel Löffel." This spoon is taken over each portion of the vaccinated surface *once* only, so as to avoid, as far as possible, admixture of blood; and by this means all the epithelium which has undergone vesicular changes, caused by the action of the specific virus, is removed in long strips of about one-eighth inch wide. Compression forceps are not needed, and the whole operation is completed in a few minutes.

Preparation of Lymph.—The whole mass of epithelial tissue removed by the spoon is collected and emptied into a glass Petri dish, and afterwards it is weighed in a delicate balance. Seven times the weight of cold boiled water and a similar quantity of glycerine are then weighed out separately. A small portion of the water is added to the dish containing the tissue scrapings, and after being stirred together the mixture is passed between the small porcelain or glass rollers of a mixing mill, invented by Dr. Doering.

Description of Dr. Doering's Lymph-Grinding Machine

The apparatus consists of an iron frame, resting on a metal base, and carrying four unsealed glass rollers. These are easily removed and sterilised, but care is taken that each roller is replaced in its original position. The spindles lie in slots, and by means of springs the rollers are pressed against each other. The rollers are

made to revolve by cog-wheels attached to the spindles on the exterior of the frame. The whole system is set in motion by turning the wheel No. 3 with the handle (which is screwed to its spindle) in a direction *from* the operator.

The mixture, lymph-pulp and glycerine, on being poured between the upper two rollers soon becomes distributed over the lower system as well. After it has reached roller No. 1, it is scraped off by a glass plate placed below, the edge being pressed against the rollers by a spring. From the plate the lymph emulsion flows into a square porcelain dish provided with a spout. To prevent the lymph spreading to the ends of the rollers, glass guides are attached to rollers Nos. 2 and 3, which conduct the emulsion towards the centre.

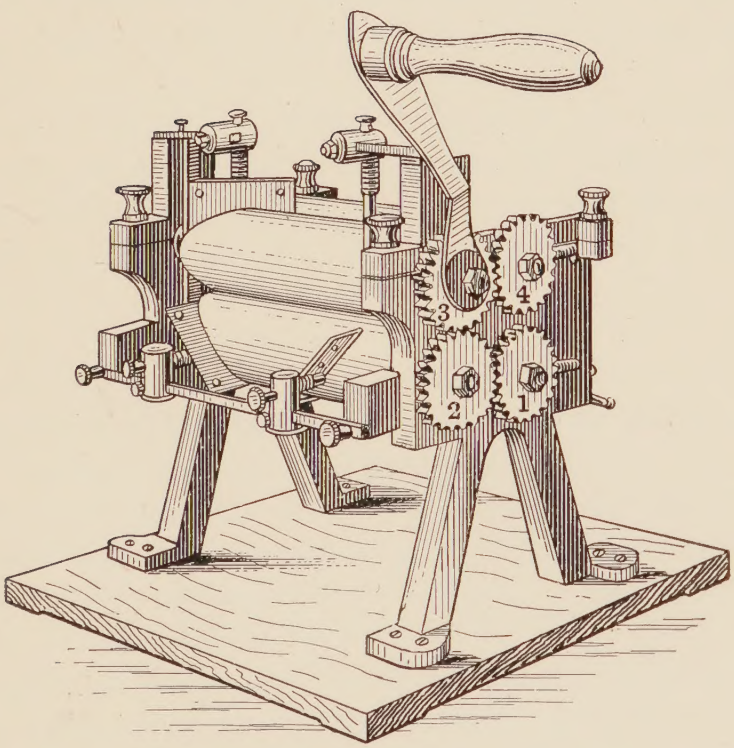
The method of working is as follows :—

After loosening the grips, right and left, fold back both arms and remove rollers Nos. 3 and 4. Unscrew both glass guides and lift out the bridge together with rollers Nos. 2 and 1. Rollers, glass guides, and glass plate are then sterilised, which process may be accomplished without withdrawing the spindles or removing the glass guides from their clamps.

The apparatus can be put together again in a few minutes. To do this, first replace the glass plate and then rollers Nos. 1, 2, and 4. Next press roller No. 3 gently back into position. Then fold over both arms, and finally fix the bridge and guides by means of the side-screws.

The mixture of lymph-pulp and glycerine is now poured little by little between rollers Nos. 3 and 4, the larger shreds of epithelium being taken up and placed in position by means of forceps. A second person turns the handle, and, in a short time, the mass, now more or less finely

PLATE XI.



Lymph-mixing machine of Dr. Döring, Berlin.

triturated, appears on roller No. 1, from which it flows into the porcelain dish placed beneath. After the whole quantity of material has passed through, the resulting emulsion is stirred with a sterilised glass rod, more glycerine is gradually added, and the mixture is then passed a second and, if considered necessary, a third time through the machine. The lymph after such further passage through the mill forms a homogeneous emulsion, the individual particles in which are of exceptional fineness.

The loss of material during preparation is exceedingly little, and the working up of the produce of a calf, amounting to about 20 grammes, can be completed in twenty minutes.

Formerly, it was the custom at this station to add both glycerine and water to the epithelium partly before and partly during its passage through the mill.

For some months past, however, it has been the practice not to add the glycerine until *after* the material has been twice passed through the mill. This grinding process is effected with more difficulty in the absence of glycerine; but the reason for the alteration is that much of the lymph is now centrifugalised,[1] a method of procedure which would be unduly prolonged if the specific gravity of the emulsion operated on had previously been increased by the addition of glycerine. The centrifuge at present in use is a small two-armed instrument worked by hand; its use involving the employment of an extra assistant for at least a couple of hours. At the end of this time

[1] This process, which was only tentative, has now (1898) been in great measure abandoned.

the minute shreds of epithelium contained in the mixture have settled into a compact mass at the bottom of the tube, and the supernatant fluid is only very slightly opalescent. This is decanted off, and an amount of glycerine equal in weight to that of the water previously employed is intimately mixed with it, after which the resulting "lymph" is stored in a stock bottle fitted with an indiarubber stopper and cap; or it is put up in small glass tubes of 1 cc. capacity, each of which contains, according to Dr. Schultz, sufficient material for 100 vaccinations. The amount of vesicle pulp collected from a single calf varies from 10 to 15 grammes. This, when intimately mixed with the usual amount of dilute glycerine, is calculated to provide sufficient material for the vaccination of, at least, 15,000 persons.

This process of centrifugalisation is as yet only tentative and experimental. The appearance of the "lymph" is thought to be improved by its adoption, and, when tested by the method of plate cultivation, it is found to be freer from "extraneous" microbes than is an equivalent amount of the emulsion when tested before treatment in the centrifuge. Objection, however, to the employment of the method might be based on this freedom if, as there is every reason for believing, the microbe specific to vaccine is present in a far greater amount within the cells of the vaccinated dermis than in the intercellular lymph spaces. Even if free in the fluid portion of the mixture, and of exceptionally minute size, the continued action of the centrifuge must tend in time to remove them, just as is found to be the case with other microbes which may be present.

The amount of glycerine and water employed in the preparation of vaccine material has been considerably

decreased during the past twelve months, the relative proportions being at present :—

Epithelial pulp	1 part
Glycerine	4 parts
Boiled water	4 parts

All "lymph" is now tested bacteriologically by means of plate cultivations, before being distributed. This is done in consequence of the recommendation of a scientific committee of which Professor Koch was a member and which has recently been sitting at Berlin to inquire into the whole subject of the collection, purification, and preservation of vaccine lymph.

Season of Calf Inoculations.—Inoculations are only carried out in the months of May, June, and July. The calves being themselves vaccinated with stored glycerinated lymph, it is not necessary to keep going a continuous series ; and in these three months sufficient lymph is manufactured for use during the whole year, throughout one of the largest of the eight districts into which the kingdom of Prussia is divided for vaccination purposes.

Disposal of Calves.—After collection of lymph, the calves are sold to the Jewish Rabbi to be slaughtered for food. We were informed that a *larger price* is given for them than is ordinarily the case with calves brought to the Central Meat Market, owing to the fact that they are in such fine condition as the result of good feeding while at the station.

Glycerinated calf lymph, collected and prepared as above stated on the occasion of our visit on 10th January 1897, was used by Dr. Cory for the vaccination (*a*) of thirty children on 19th January, with an insertion success of 97.1 per cent ; (*b*) of six children on 21st January, with an

insertion success of 86.6 per cent; (*c*) of forty children on 25th February, with an insertion success of 92.3 per cent; and of thirty-three children on 4th March, with an insertion success of 67.5 per cent. This sample of glycerinated lymph was again used after having been kept until six months had elapsed since the date of its preparation. It was then found that its activity as vaccine lymph had practically disappeared. It is right to state in this connection that at the Board's Animal Vaccine Station there are, as yet, no means of storing lymph elsewhere than in the somewhat high temperature at which the operating rooms are maintained.

Dresden

The Animal Vaccine Institute, of which Dr. Chalybäus is director, is situated in the northern suburbs of Dresden. It consists of a small two-storied building, containing, on the ground floor, an operating room and three other rooms, while the whole of the first floor is utilised as a dwelling for the caretaker. Adjoining this building is a small stable containing stalls for the calves.

Dr. Chalybäus informed us, however, that the Animal Vaccine Institute was hardly arranged in accordance with modern requirements, having been established more than twelve years ago, when the present methods of preparing lymph were not in vogue.

The stable in which calves are placed on arrival contains two stalls and a tank bath in which the animals are thoroughly washed before use. The calves are lifted into and out of this tank by means of belly-bands attached to a system of pulleys fixed to the ceiling. After having been dried with cloths they are vaccinated and then placed in another stable on the opposite side of the house.

The calves are bedded in their stalls on fine wood shavings, which are said to have the advantages of being clean, dry, and comfortable.

The operating room is about 20 feet square, and contains two tables. One of these is for calves ; the other, of larger size, and fitted with mechanical arrangements for tilting and raising, is for young bullocks, which are occasionally employed for purposes of vaccination, when either an extra amount of lymph is required or when it has been impossible to obtain the required number of calves.

By preference, Dr. Chalybäus employs cow-calves of from six to eight weeks old.

The calf table is an oblong, shallow trough of wood, provided with straps and with *two* iron uprights at one end, to which the hind limbs of the calf are fixed in a V-shape. This method of fixation, however, enables the animal to struggle to such an extent as to raise its hind-quarters completely off the table.

After having been shaved, and before vaccination, the animal's skin is washed with soap and hot water containing lysol. The soap suds having been washed off with more water, and the skin dried with a cloth, benzine is poured over the surface to render it more aseptic, and is rubbed in with sterile sponges of gauze, which are kept for use in a sterilised glass-stoppered bottle. Lengthy incisions are next made with a blunt scalpel, in the long axis of the body, over the inside of the thighs and over the whole surface of the abdomen from the vulva to the umbilicus ; also over the lower ribs. Glycerinated lymph *which has been stored in sealed tubes for from three to as long a period as eighteen months* is next rubbed in over the area of the incisions with the flat surface of a small trowel-shaped instrument.

We were informed that much difficulty is experienced in this establishment in obtaining calves suitable for purposes of vaccination, as throughout Saxony it is the custom to slaughter these animals for food at a very early age, sometimes within a few days of birth. This being so, calves have to be imported from a distance, most of those employed by Dr. Chalybäus coming from Berlin or Hamburg. They are obtained by a local cattle-dealer, who charges twenty marks for their use, and who removes them for slaughter immediately after collection of the lymph.

Calf-to-arm vaccination is never employed in Saxony, as it is thought to be undesirable to use lymph from an animal until a necropsy has shown it to have been entirely free from disease.

Collection of Lymph.—The lymph, or rather the vesicle pulp, is collected after an interval of four complete periods of twenty-four hours. The skin is first washed with white soft soap and hot water, the operation being carried out with the aid of a large house-painter's brush. Such crusts as have formed are removed as far as possible with the edge of an ordinary metal teaspoon, after which glycerine is poured over the skin and rubbed in with gauze sponges.

The pulp is collected by scraping with a Volkmann's spoon, but as Dr. Chalybäus goes over the same surface again and again, a not inconsiderable amount of blood becomes mixed with the epithelial scrapings. The raw surface of the abdomen is afterwards dusted over with fine oatmeal.

The pulp thus collected is weighed and is then run through a mixing machine invented by Dr. Chalybäus, of which a special description, written by him, together with an illustration, is appended to this report. The necessary

motive power is supplied from a small water motor fixed beneath the floor of the room.

After being ground up in the machine, four times the amount of a mixture of glycerine and sterilised water (water 3 parts, glycerine 1 part) is added to the vesicle pulp, and the whole is then run through once again to ensure thorough admixture. The resulting emulsion is received into a porcelain mortar placed beneath the machine. The mortar is removed when all the material has passed through, and its contents are then taken up by suction into tubes of somewhat large calibre which, when filled, are closed at either end by means of sealing wax.

Dr. Chalybäus considers that 1 gramme of glycerinated emulsion is sufficient for 80 vaccinations. The vesicle pulp obtained from a single calf affords from 50 to 75 grammes of the glycerinated emulsion; or, in other words, enough for the vaccination of from 4000 to 6000 persons.

Glycerinated calf lymph, collected and prepared by Dr. Chalybäus on the occasion of our visit on 12th January 1897, was used by Dr. Cory for the vaccination of 15 children on 21st January. The children were, as usual, vaccinated by means of five insertions, and every insertion gave a successful result.

[A further sample of lymph collected on 12th January 1897 was employed by Dr. Cory nearly six months later, for a further series of vaccinations. As a result he again attained complete insertion success. The lymph examined bacteriologically at this date by the method of plate cultivation was found to be completely free from extraneous micro-organisms.—S. M. C.]

(*Translation.*)

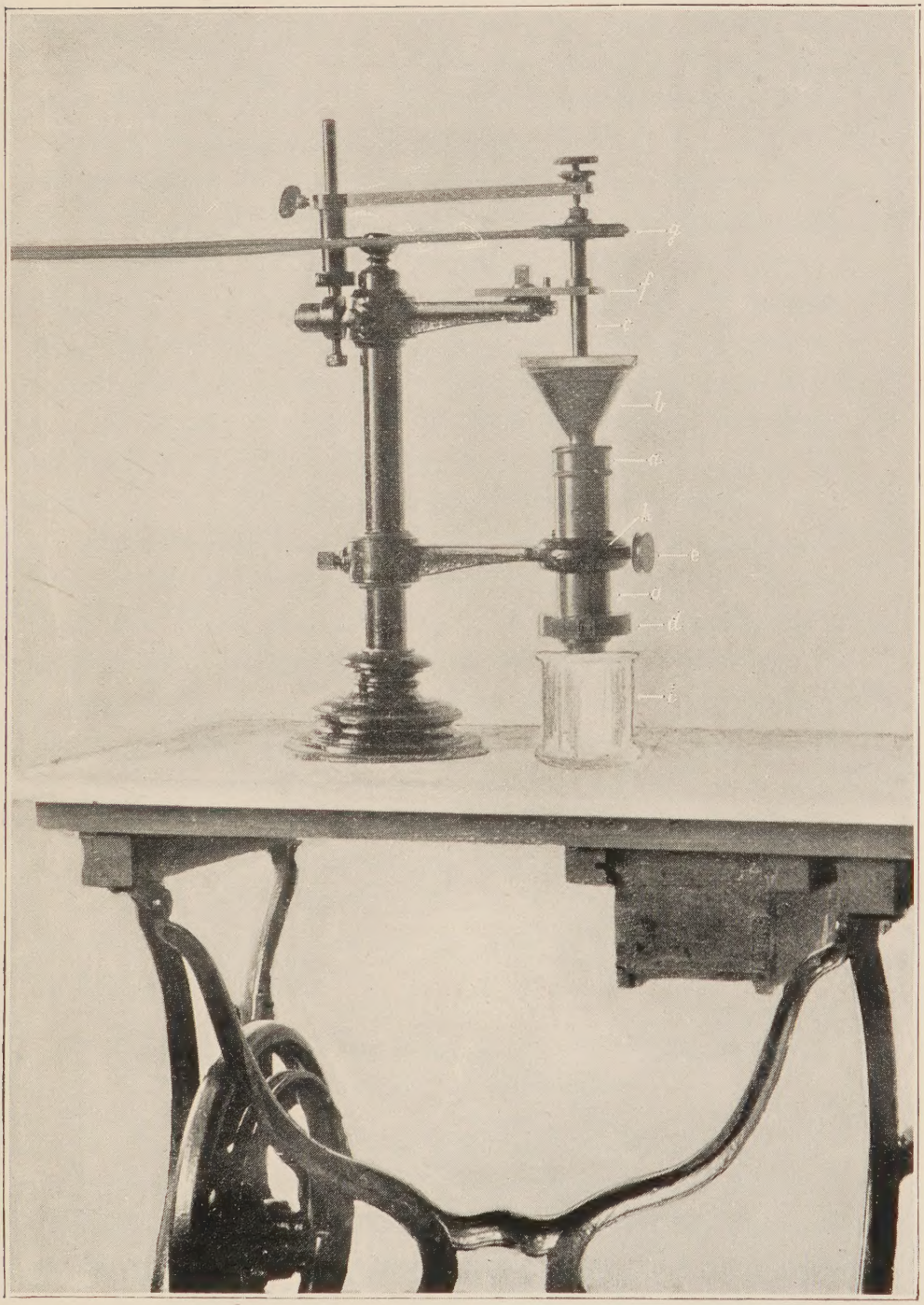
Report of the Royal Institute for Vaccination in Dresden.

About the Technique of Preparing Animal Lymph, by Dr. Chalybäus, Dresden.

As a rule, the exclusive use of animal lymph for vaccination has been everywhere adopted, whilst the use of human lymph, taken from children, has been almost entirely abandoned, and public institutes for vaccination prepare only calf lymph. The preparation of animal lymph needs special contrivances, because the lymph scraped out of the "smallpox of a calf," in order to make it available, must be triturated and ground to a fine pulp, and with the addition of glycerine, turned into a thin and homogeneous emulsion. When triturated in a dish or bowl, this operation takes, for the quantity of lymph obtainable from one calf, three hours' time, and cannot therefore be done by the physician himself; nevertheless, great care must be taken, as it is unwise to leave that part of the preparation of lymph to a common workman, who cannot be properly and continuously watched.

As far back as 1889 I constructed a machine for triturating lymph, which stood the test of use in the Institute of this country and of those of many others. In 1893 the machine was improved. It is now fitted upon the marble table of a sewing engine, and moved either by foot or, still better, by means of a small hydraulic, steam, or electric motor.

A cylinder formed out of two equal parts, having in its inside threads of a screw, is attached to the shank of a column; a close-fitting spindle, likewise provided with threads of a screw, turns in the cylinder. Through a



Lymph-mixing machine of Dr. Chalybäus, Dresden, as modified for use in the laboratories of the Local Government Board.

funnel the raw lymph is put into the upper end of the cylinder; in turning the spindle the lymph is rubbed and ground to the utmost, and leaves the cylinder on the lower opening, dropping into a glass dish.

By means of this contrivance the preparation is completed in about fifteen minutes, although the work is done in a far better way than as usually in a dish. The lymph, consisting of coherent and liquid parts, is thoroughly rubbed, not only squeezed, and turned into homogeneous emulsion. It does not lose its natural colour, nor does it get warm.

The apparatus is made of steel bronze, and its parts, comprising cylinder, spindle, and funnel, can easily be separated and disinfected in boiling water or otherwise.

The trituration occurring whilst the apparatus is closed—the opening of the funnel is closed by a cover of glass—no dust can enter, and should a calf hair accidentally have dropped into the lymph it can be removed whilst the lymph slowly drops out of the cylinder. No loss of lymph can occur, because the machine retains none of the material with which it is fed.

The machine can be obtained through the Royal Institute for Vaccination, in Dresden, price 200 marks. Every machine is tested by the president of the above-mentioned institute, and a certificate is granted to each one sent out, as a proof of its fitness.

Directions for using the Machine for triturating Lymph, invented by Dr. Chalybäus

1. *To take the machine to pieces*, slack the screw in claw *f* on which the driving-wheel *g* and spindle *c* are attached. Open the claws and remove the spindle and driving-wheel *g*, taking care not to let the former drop. Take off funnel *b*, slack screw of lower clamp *d*, and unscrew the middle

clamp *e*, holding the cylinder in the left hand. Take the cylinder out of the support *h*. In order to separate the two parts of cylinder, use a small piece of wood by inserting it in the hollow, but care must be taken not to drop the parts when separated. Remove the driving-wheel from the spindle, and clean it, as well as the spindle and funnel, with a brush, and sterilise in boiling water.

2. *To put the machine together*, first join both parts of the cylinder, put on the lower clamp and turn its screw a little, pass the cylinder vertically through the support, and place the funnel on the top of the cylinder. Then screw the driving-wheel on the upper part of the cylinder, open the claws and insert the spindle so far that the claws grasp the spindle close under the driving-wheel, shut the claws, and screw tight. Bring the lower ends of spindle and cylinder exactly in a line and fasten the lower clamping screw. Finally, adjust the belt on the driving-wheel.

3. *To put the machine in motion*, move the fly-wheel *k* outwards, and to avoid friction use vaseline for lubricating the moving parts. It is advantageous first to run some glycerine through the cylinder, after which the lymph is placed in the funnel and the cover adjusted. If preferred, however, glycerine can be added to the lymph, and the mixture passed through the machine. The finished lymph leaves the lower end of the cylinder and drops into a small glass vessel *i*.

COLOGNE

The buildings provided for this Institution are the most recent of their kind in Germany, and, as we were informed previous to our visit, all the fittings are of the most modern description.

We visited the Institution with Dr. Vanselow, where we

were also met by the assistant director, the veterinary surgeon attached to the staff, and certain other gentlemen.

Dr. Vanselow presented us with a reprint of a paper written by him, showing a description of the buildings, illustrated with blocks giving the elevation and ground plans.[1] We append a translation of this, so that it is unnecessary here to enter into any details as to structure.

Stable.—The calf stable, which adjoins the collecting room, contains ten stalls, one of which is reserved for any calf used for experimental purposes, while another is merely a pen forming the platform of a weighing machine. The sides and ends of the stalls are formed solely of a series of iron bars, painted grey, and they are of such narrow width as to make it impossible for a calf to turn round, it being thus prevented from licking the inoculated area of its abdomen. Each stall is provided with a gate at either end, opening outwards, and bears a numbered label of iron. The flooring of the whole stable is formed of cement concrete, over which in each stall is placed a wooden rack or platform which is raised about 3 inches from the floor. These racks are formed of wooden splines about 2 inches square, placed close together, the upper edges of each spline being slightly rounded. On these racks the calves stand or recline, no bedding of hay or straw being employed.

Calves.—The calves range usually from about six to eight weeks old. They are purchased in the meat market which immediately adjoins the establishment. The calves required for the current week are bought on Monday, and they are sold on the following Saturday, after their slaughter and the collection of lymph on the previous day; so that the stable is always empty from Saturday to the following Monday. The calves are kept under observation in the

[1] Plans not reproduced here.—S. M. C.

stable for twenty-four hours after their reception, and are vaccinated on Tuesday. Only three complete days are allowed for the progress of the local results of vaccination. On Friday the animal is slaughtered in a small slaughter-house opening off from the stable, and immediately it is dead the carcase is brought into the collecting room on a trolley, the abdomen washed, and the epithelial pulp of the vaccination area is removed by means of a sharp spoon.

Vaccination of Calves.—We did not see the process of vaccinating the calves, but we learnt that it was carried out in an exactly similar fashion to that employed at the Berlin and Dresden stations, namely, by long parallel incisions over which glycerinated lymph is rubbed by means of a spatula or other flat-bladed instrument. The lymph employed for the vaccination of calves is always kept for a period of at least six weeks after glycerination, in order to ensure that it shall be as free as possible from extraneous organisms before it is used to vaccinate the calves.

After collection of the lymph pulp, and while it is being prepared for use, the carcase of the calf is taken back to the slaughtering room, where it is skinned and opened. The internal organs are removed and brought in on trays to be examined by the veterinary surgeon. In the event of his forming the opinion that any of the organs presented any condition indicative of disease, the lymph derived from the animal in question would be at once destroyed.

In view of this precautionary measure it is not deemed necessary to test the calves by the injection of tuberculin prior to their vaccination.

Collection and Preparation of Lymph.—The greatest amount of vaccine is collected during the months of March, April, and May, when from six to eight calves are employed

every week. For the remainder of the year the weekly vaccination of one or two calves is found to be sufficient to supply all the lymph required for human vaccinations and revaccinations in the Cologne district.

In the preparation of the lymph material the epithelial pulp from the vaccinated area is removed by scraping with a Volkmann's spoon, and is received in a small glass dish. In this it is weighed, after which it is turned out into a mortar and thoroughly triturated; at first without any addition of water or glycerine; later, small quantities of water are gradually added to the extent of *five* times the weight of pulp. The mixture having been ground up still further, double the quantity of glycerine is finally incorporated. Thus, at the time of our visit, 10 grammes of pulp having been collected from one calf, the composition of the finished emulsion was as follows:—

Pulp	10	grammes
Water	33·5	,,
Glycerine	66.5	,,

Dr. Vanselow informed us that this quantity would suffice for the vaccination of 5000 children.

The emulsion is afterwards forced into small bottles and tubes by means of a machine actuated by water power. This machine, which is manufactured by a Vienna firm, appears to be decidedly useful and convenient, and is capable of being worked in connection with any form of pressure apparatus.

The small bottles are of different sizes, and contain lymph sufficient for 50, for 100, and for 150 vaccinations respectively. The bottles and their corks are all of them sterilised prior to use.

We were struck with the numerous precautions which

are taken in this institution to ensure thorough asepsis throughout the various stages of lymph production. Thus, in the collecting room, the flooring is of cement concrete, the walls are lined internally for about half their height with opaque glass tiles, the upper half being of Parian cement. The shelves are of glass supported on iron brackets, and the surface of all tables consists of thick slabs of glass over green cloth, the glass being removable if necessary. The institution is furnished throughout with electric light, and sterilised hot water is supplied as needed from a small apparatus affixed to the wall of the collecting room. Indiarubber pipes used in connection with the hot-water apparatus and the pressure apparatus employed for filling tubes are kept in a strong solution of carbolic acid when they are not in use. The director and all his assistants wear linen covers over their clothes, but only the sleeves, which are detachable, appear to be sterilised prior to each occasion on which they are used.

On the first floor of the building is a bacteriological laboratory, which is reached from the collecting room by an iron spiral staircase. It is fitted up with an autoclave, incubators, etc. But owing to the director being engaged in private practice, he has no time to work in this laboratory, which is, therefore, only used when it is desired to sterilise cloths, instruments, or glass-ware.

With the lymph and pulp material collected and glycerinated in our presence on 16th January 1897, Dr. Cory vaccinated thirty-four children on 19th January, with an insertion success of 98.8 per cent, and twenty-one children on 11th February, with an insertion success of 93.3 per cent.

(*Translation*)

THE ROYAL LYMPH STATION FOR THE RHINE PROVINCE AT THE NEW CITY CATTLE AND SLAUGHTERING ESTABLISHMENT, COLOGNE, BY SANITARY COUNCILLOR, DR. VANSELOW, DIRECTOR.

The public institution for the preparation of animal lymph to meet the needs of the Rhine Province and the Hohenzollern districts was erected in Cologne in 1889. The rooms at the old slaughtering establishment which were used until 1895 were extremely defective, being narrow, damp, and dark, and rendered any practically uniform arrangement impossible. In building the new cattle and slaughtering establishment, the provision of a suitable annexe for the production of lymph was borne in mind from the very first; and so the present institution originated, which meets all the demands of hygiene, and may justly be regarded as the prototype of such institutions.

The annexe is situate at one end of a large cattle shed, both having a common partition. The main front faces the north, so that all the rooms, as there are windows only in the front, receive their light equally distributed from that direction. The building is very solidly built, but undercellared only, and to the smallest extent, on the eastern side. As, however, the whole of the ground was filled in, and only absolutely dry and permeable material employed, there is no fear of any dampness of the rooms.

On the ground floor of the building are the corridor, collecting room, calf stable, slaughter room, doctor's room, office, and a closet.

On the first floor, which is reached from the collecting

room by a convenient winding staircase, is the laboratory, adjoining which, on both sides, is a large garret. The entrance to the building is from the street, and so arranged that the cattle establishment has not to be traversed in order to enter; the wall belonging to the entrance being continuous with the wall which encloses the cattle establishment. The calves are driven into the station through the doorway of the slaughtering room, which lies at the opposite end of the building.

While the floors of the slaughtering room, calf stable, collecting room, and laboratory are of concrete, the doctor's room and the office have inlaid wooden floors (parquetry); the corridor and closet are laid with "Mettlacher" tiles; and, finally, the rooms in the roof (garrets at the sides of the laboratory) are laid with floor boards. In the slaughtering room, calf stable, and collecting room the floor is slightly sloped in one direction, and at the lowest point there is a drainage outlet which is shut off by a small intercepting trap. The height of the rooms on the ground floor is $3\frac{1}{2}$ metres, except the collecting room, however, which is $4\frac{1}{2}$ metres in height, and that of the laboratory is $3\frac{1}{2}$ metres. All the rooms on the ground floor are vaulted with plain solid arches. The laboratory and garrets have rafters and wooden ceilings. The collecting room, calf stable, and closet are lined, to the height of $1\frac{1}{2}$ metres from the floor, on all four sides, with white opaque glass tiles; the slaughtering room, on three sides (the door side is excluded), to the same height with white glazed tiles. The remaining part of the walls in these rooms is painted with white porcelain enamel. The walls in the doctor's room and the office are papered. In the laboratory and corridor the walls are painted with oil paint. The collecting room is brightly lighted by a window 4 metres wide by 3 metres

high. The light entering becomes strongly reflected by the brilliant white walls. The laboratory has three windows, the calf stable two, and the doctor's room and office one large window each. The closet and slaughtering room have each one small window. All the rooms are, therefore, amply lighted.

The calf stable contains eleven stalls for the reception of the calves, *i.e.* six on one side and five on the other side of the centre passage. The stalls are so constructed that the wall nowhere forms the boundary of a stall, and a clear passage is thus given all round the stalls; they are each 70 centimetres wide and 150 centimetres long. The enclosures are formed of iron lattice-work; at the two narrow sides of each there is a door permitting the calves to be taken in or out by either side according to convenience. The iron lattice-work is painted a light grey colour, so that every speck of dirt can at once be seen and removed. On the floor of the stalls lie wooden gratings. Upon the space which would correspond to the twelfth stall a weigh-bridge is sunk, enclosed with the above-described iron work; this machine allows of the calf being weighed while being taken through. The hollow in which the weigh-bridge stands has also a smell-preventing arrangement. The ventilation of the stable is obtained by a large tube which passes through the garret above. The collecting room has a flap ventilator in the window, as shown on the plan. Between the calf stable and collecting room there is an arrangement of double doors, one of which is thickly padded. The doors, padding, and through-air draught isolate the collecting room both from smell and noise. The closet is a so-called "Unitas" closet. Water is supplied by the city main, and in every room a sink is provided. The lighting is by electricity; in the laboratory

however, the "Auèrches Glühlicht" (a form of incandescent light) has been found preferable.

The heating is effected by means of American stoves; for the doctor's room, however, a gas stove has been provided by reason of its greater suitability. Large gas stoves serve for the heating of the water and milk. For the storage of considerable quantities of lymph a sufficiently large room is reserved in the refrigerating house of the city cattle establishment.

The furniture provided for the institution is worthy of the handsome rooms, and consists throughout of oak. The laboratory is completely fitted for bacteriological investigations, containing all sterilisation apparatus—thermostats, an excellent microtome, microscopes, centrifuge, etc.

The extent of the lymph production may be understood from the following figures:—In the year 1894 about 356,000 portions of lymph were issued, and in this year (1895) the number of portions will nearly reach 400,000.

GENEVA

The Institut Vaccinal Suisse, which was visited by one of us (Dr. Copeman) only, is situated at Lancy, on the outskirts of Geneva. It was founded in 1882 by M. Charles Haccius, the present director of the establishment. Originally a private venture, it is now recognised by the various cantonal Governments, M. Haccius, in consideration of an annual subvention, supplying to public vaccinators throughout Switzerland, free of cost, all the lymph required by them in the performance of their duties.

The building in which the Vaccine Institute is housed adjoins a model dairy, also established and carried on by M. Haccius. The institute building contains two stables

for calves, an operating and collecting room, a laboratory, a room in which the packing and distribution of the lymph are carried out, and the director's room.

Stables.—Each of these contains four stalls. The side walls of the reception stable are of concrete; those of the stable adjoining the operating room are of wood. The floors of both stables are of concrete. The stables are kept at a temperature of 18° C. to 20° C. Calves are received into the first-mentioned stable, and are there kept under observation for four or five days, at the end of which period they are passed into the stable next the operating room. The bedding of the stalls consists of fine wood shavings, this material being, in the opinion of M. Haccius, decidedly preferable to straw as regards both the cleanliness and comfort of the calves.

Calves.—These range usually from about three to four months old. They are fed on milk, obtained direct from cows in the adjoining dairy, in addition to which they are allowed oatmeal and a certain number of eggs, but no hay. The calves are purchased from peasants in the surrounding districts. After vaccination and collection of the lymph, they are sold to a butcher in Geneva, at a loss of about £1 on each calf. They are slaughtered in the public abattoir, and the veterinary inspector attached to that establishment furnishes a certificate relating to the healthiness of the calf and the condition of the viscera as ascertained on examination of the carcase.

Vaccination of Calves.—For the purpose of vaccination the calf is strapped down to a tilting table, similar in its main features to those employed in England. The head of the animal is covered with a leathern mask. The whole of the abdomen, the inside of the thighs, and a considerable area of the right side of the body of the animal are shaved, white soft soap and hot water being used in the process.

The skin is next washed with solution of lysol (2.5 parts per 1000), and finally with hot boiled water. It is dried with sterilised gauze sponges. The actual vaccination is carried out in a manner similar to that universally employed in the German Government establishments already described, with the exception that the parallel lines of incision are discontinuous at intervals of about four inches. Occasionally a certain number of incisions are made at a greater distance from each other, and only about a couple of inches in length, in order that the condition of the resulting vesicle may be more readily observed. Any blood which exudes from the incisions is removed with sterilised gauze sponges, and then the skin is put on the stretch, while glycerinated lymph is rubbed into each incision by means of a small and thin ivory spatula. The lymph employed consists of one part of vesicle pulp incorporated with two parts of undiluted glycerine, and the resulting mixture is stored for about a month prior to use.

Collection and Preparation of Lymph.—After the lapse of four days and a half from the time of vaccination, the calf is again placed on the table, and the vaccinated area washed with warm boiled water without the addition of any antiseptic. After drying with sterilised gauze sponges, the vesicle pulp is removed by scraping with a sharp spoon. The resulting pulp is collected in a glass pot provided with a cover, and when all has been removed the total amount is weighed. Sufficient glycerine (undiluted) is then added to cover the mass of pulp, and the vessel and its contents are set aside for a few days. Subsequently, glycerine and water are added in proportions requisite for attaining the following standard :—

Vesicle pulp	1 part
Glycerine	2 parts
Water	1 part

and the mixture is then thoroughly triturated in a mixing machine of the kind invented by Dr. Chalybäus of Dresden. The resulting emulsion is employed for human vaccinations, the "seed material" used for the vaccination of calves, having, as already stated, no water added to it.

Occasionally clamp forceps are employed in the collection of lymph from the smaller vesicles, when it is required to store it along with glycerine in fine capillary tubes; the resulting material, containing comparatively little epithelial tissue, being therefore more readily drawn up into the tubes. When collection is carried out in this manner the "crust" is first removed from the vesicle, which is then gently scraped with a lancet. The material thus obtained is mixed with glycerine in the usual fashion.

The emulsion, which is never sent out for use until at least four weeks after collection of the vesicle pulp, is stored prior to distribution in large glass-stoppered tubes. It is sent out in flacons, plaques, and capillary tubes, according to the amount required in any given case. The flacons, small glass tubes made of amber-coloured glass and provided with corks, are of sufficient size to contain enough emulsion for 25, 50, and 100 vaccinations respectively. The plaques consist of two small squares of glass, one of which has a shallow excavation on one surface. This is filled with emulsion, then covered with the plain square, and the edges sealed with paraffin. Quantities of emulsion sufficient for five or ten vaccinations are sent out in this way; while fine capillary tubes, which are sealed with paraffin, are used for sending out lymph for the vaccination of one person only. All flacons, plaques, and tubes are sterilised before being filled. In order to send them safely through the post they are enclosed in neat metal cases differing in size and shape. These, together with

certain printed matter, including a card to be filled up in accordance with the results obtained from use of the contained lymph, are enclosed in a stout glazed orange-coloured envelope secured with a metal clip.

M. Haccius stated that he had, especially of late, experienced some difficulty in getting public vaccinators to fill up and return the cards sent with each consignment of lymph, so that it was not possible to obtain full statistics as to the success attending the use of the lymph sent out from the Institute. On looking over with him, however, a number of cards which had come to hand within the last few months, it appeared that in all cases the success attained was very great; in a not inconsiderable proportion the *insertion* success had reached 100 per cent.

M. Haccius further stated that the structural arrangements of his Institute were the same as when it was first started in 1882, so that they are not in some respects such as would be considered most desirable at the present time. Nevertheless, it was impossible not to be impressed with the strict precautions taken to ensure the utmost cleanliness in the case both of the premises and of all persons employed in the various details of the work.

APPENDIX No. II

EXTRACTS FROM THE TRANSACTIONS OF THE SEVENTH INTERNATIONAL CONGRESS OF HYGIENE AND DEMOGRAPHY: LONDON, 1891. VOL. II. SECTION II. pp. 319-26.

The Bacteriology of Vaccine Lymph: by S. Monckton Copeman, M.A., M.D. Cantab., Research Scholar of the British Medical Association, late Assistant Lecturer on Physiology, St. Thomas's Hospital.

NUMEROUS have been the attempts, ever since bacteriology first took rank as an exact science, to solve the problem as to the nature of the active principle of vaccine lymph, and in consequence of this, and of the great difficulty of the subject, a mass of literature has gradually accumulated sufficient to appal any one newly entering upon the task.

The fact, however, that in the absence of any sufficient knowledge on this point, the whole practice of vaccination as at present carried out is more or less empirical, may perhaps furnish excuse for still further attempts in this direction. I therefore venture to bring forward the results of an investigation of this subject, on which I have been engaged at intervals for the last eighteen months.

As a first step it appeared desirable to obtain proof, if possible, of the particulate nature, or otherwise, of the essential cause of vaccinia.

. . . . . .

I next turned my attention to the identification of the

various micro-organisms which can be grown as the result of inoculation of various nutritive media with vaccine lymph. And here I should remark that in all the experiments of this series, calf lymph alone was used, as it seemed probable that by these means one would obtain more constant results.

. . . . . .

As it was evident that more than one organism could usually be obtained by the inoculation of lymph into nutrient media, plate cultivations were made, with the object of separating out the different varieties present, both gelatine and glycerine agar being used for this purpose. In this manner I succeeded in obtaining pure growths of various micro-organisms, of which those which almost invariably occurred included micrococci, apparently much resembling, if not identical with, the following :—

(1) M. Pyogenes aureus ;
(2) M. Cereus flavus ;
(3) M. Epidermidis (? Staphylococcus albus, Klein).

In these observations I find my experiments are in agreement with those of Pfeiffer, while doubtless these various micrococci correspond respectively with Buist's so-called orange, yellow, and white vaccine.

. . . . . .

As it appeared possible that their exuberant growth might be capable of preventing the development of the more important organism for which I was seeking, an attempt was next made to isolate it by some method of treating vaccine lymph, which should destroy all extraneous micro-organisms without injuring its potency for vaccination.

Having in mind the method by which Kitasato succeeded in obtaining pure cultures of the tetanus bacillus, I con-

sidered whether it might not be possible, in like manner, to isolate the specific organism of vaccinia. For the purpose of testing the effect of similar measures, I exposed capillary tubes of fresh vaccine lymph to different temperatures for varying lengths of time, afterwards inoculating a portion of the contents of these tubes on the calf, a second portion being used for the making of plate-cultivations in nutrient gelatine and agar-agar. By proceeding in this manner, after numerous experiments, I presently arrived at a temperature at which those organisms which usually grow so luxuriantly when vaccine lymph is inoculated into nutrient jelly, are apparently incapable of continued existence after exposure to its influence for a certain length of time.

. . . . . .

After a long series of experiments with lymph exposed to various temperatures between the limits mentioned, and for varying periods of time, I have apparently determined that the required temperature is one ranging between 38° C. and 42° C. For exposure to the higher of these temperatures for an hour has the effect of preventing the growth in plate-cultivations made subsequently, while at the lower temperature a few points of growth are occasionally seen after the lapse of a day or so. At the same time, however, the higher temperature appears occasionally to exert an injurious effect on the lymph, as far as regards the normal vesiculation which should result from the inoculation of vaccine lymph. Further experiments are therefore needed before the most suitable temperature and most desirable length of exposure can be definitely determined.

. . . . . .

Early in my experiments, my attention was called to the fact that lymph stored in capillary tubes nearly always

becomes cloudy after a longer or shorter interval, while at the same time it often becomes uncertain in its action when subsequently used for vaccination.

I endeavoured therefore, in the first place, to determine the reason for the opacity which occurs in stored lymph; and, secondly, to find, if possible, some means of preventing such an occurrence. As regards the first point, experiments were carried out as follows:—A large number of capillary tubes were filled with calf lymph, every precaution as to cleanliness and careful sealing of the tubes being observed. These tubes were then set aside, not, as is usually the case, lengthwise, but on end. After the lapse of a few weeks all the tubes presented little points of opacity, and on careful examination it was obvious that each of these occurred where a surface of the lymph was in contact with a bubble of air. Moreover, it was always at the *lower* end of the line of lymph that such opaque points were found, thus showing that they were composed of something possessing a higher density than the lymph itself, as otherwise there was no apparent reason why they should not also be met with at the upper limit of each thread of lymph where air was also present.

This appearance was quite independent of any coagulation in the lymph; indeed, on breaking some of the tubes in which it was most marked, no coagulum was found. On the other hand, where clotting had taken place after the lymph was stored, the opacity was often not in discrete points as in the other tubes, but formed with the coagulum a central whitish thread in the midst of a clear fluid. In these cases, the tiny particles of which an opaque point was obviously composed had apparently become more or less entangled with the fine thread of fibrin which had resulted from the process of coagulation.

Cultivation experiments were then carried out, gelatine tubes being inoculated from opaque points in stored tubes, and also, as a control, from tubes of comparatively fresh lymph, the capillary tubes used in these experiments having been sterilised previous to their being filled with lymph. In each case, plate-cultures were made from dilutions of the gelatine tubes first inoculated. As a result, many more colonies were found in the plates poured from inoculations of the old tubes than from those descended from the fresher lymph. We are therefore apparently justified in considering that the opacity of old-stored lymph is, in the main, the outcome of an enormous multiplication of aërobic bacteria, the ancestors of which are present in the lymph when first taken, although their numbers are then so comparatively small as not to render it in any way turbid.

As I have already shown, a certain amount of heat, when applied to lymph in sealed capillary tubes, inhibits the growth of these aërobic bacteria, while a still higher temperature kills them. The exposure of freshly-stored lymph, then, to a proper temperature for a sufficient length of time, ought to prevent the subsequent appearance of opacity, and such is apparently the case. Without great care, however, there is in this method considerable danger of rendering the lymph inert.

A simpler method of obtaining the desired result, however, is found in the admixture of the lymph with a certain proportion of 50 per cent glycerine in distilled and sterilised water prior to storage in capillary tubes, which also should have previously been sterilised by heat.

Müller showed, long ago, that lymph might be diluted with three times its bulk of such a mixture and still retain its properties unimpaired, a fact which has been taken

advantage of by more than one purveyor of trade lymph. Experiment shows that not only is this so, at any rate for a considerable time, but also that, in tubes filled with such diluted lymph, opacity does not apparently result. The glycerine appears to inhibit the growth of those aërobic bacteria, which in former parts of this paper I have termed "extraneous," signifying by that term that their presence is not in any way essential, indeed, probably rather the reverse, to the successful action of the vaccine lymph. . . . Another argument, in favour of the use of lymph diluted in this way with glycerine, is found in the fact that, as before stated, such a mixture does not dry up nearly as readily as ordinary lymph, and therefore in the hands of operators, all but the most expert, affords greater facilities towards the attainment of a uniformly successful series of vaccinations. The fact also that a much larger supply of lymph would thus be available is so obvious that it needs not to be insisted on.

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